

Towards a global understanding

The programme launched in Berne this week to understand the changing environment for human beings on the surface of the Earth is laudable, but needs strong management.

WHO knows the initials IGBP, the acronym for the ugly legend International Geosphere-Biosphere Programme? In spite of the enthusiasm at this week's meeting of the International Council of Scientific Unions at Berne, some time will pass before the name is familiar. One snag is money: many national research enterprises are so short of funds to support bread-and-butter projects that they will ask for more only hesitantly and therefore unconvincingly. The implied hope that similarities with the International Geophysical year will unlock empty purses may be fruitless. Another snag is method: IGBP is a grand idea, and a timely one, nothing less than that of laying the foundations for a thorough understanding of the causes of global environmental changes of all kinds. But the execution of this ambition will not be child's play. In the absence of a peg on which to hang the programme (IGY was planned to span an impending solar maximum), national organisations will be tempted to relapse existing but not particularly significant programmes as their contributions to IGBP. The project will also be at risk of ambush by those who would dramatise local environmental problems by having them elevated to global status. The scientific committee that will guide the programme will need a clear head and a strong nerve.

The first need is for a sense of priority. The agreed starting point is that the scale of human activity on the surface of the Earth is now comparable with the scale of the still poorly understood external influences which regulate the environment. That does not concede the case, morbidly yet fondly put by many who should know better, that the human influence is already so great that we are poised on a knife-edge between disaster and its near-avoidance. The sober anxiety is merely that, in respect of problems such as the influence of atmospheric carbon dioxide on the climate, present capacity to predict the course of events is rudimentary. It is demeaning as well as dangerous not to be able to tell what even the century ahead may hold, which is a sufficient case for IGBP. The programme managers should use that principle to decide which projects they approve.

Oceans

The greenhouse problem is a good illustration of how this philosophically parsimonious principle should apply. The steady accumulation of carbon dioxide in the atmosphere has been observed with ever-greater accuracy and completeness for three decades, but the accumulation is only roughly half as fast as calculated from the known consumption of fossil fuel. Where has the other half gone? Into more vegetation, or into solution in the oceans? If the oceans are an expedient storehouse for an otherwise troublesome atmospheric absorber of solar energy re-radiated from the surface of the Earth, is it permanent or merely temporary; a means by which trouble is postponed? Worse still, why is there still no clear climatic consequence of the accumulation so far recorded, an increase of roughly ten per cent (to 345 parts per million) in only the past thirty years?

Part of the trouble lies in the crudity of even the most sophisticated climatic models used, among other things, to predict that surface temperatures will increase as carbon dioxide accumulates. The models cannot, for example, handle real clouds (which could provide stabilizing influences) rather than mere

average cloudiness, but there are probably many other features of the Earth's atmosphere that are not yet sufficiently well understood conceptually. It remains something between a puzzle and a scandal that the mechanism of the presumed high rate of transfer of carbon dioxide to the oceans has not been confidently identified after three decades of worry about the atmospheric greenhouse. And while evidence has accumulated that the Earth's climate is indeed substantially influenced by external events such as the changing parameters of the Earth's orbit about the Sun there has as yet been no convincing attempt to use this knowledge predictively, no doubt because the other uncertainties are greater than the effects expected from variations of the orbital parameters. In this confusion, it is also possible that the expected climatic changes attributable to carbon dioxide are already under way, but have gone unrecognized because nobody is sure what signals to look for in the seasonal noise.

Tall order

The atmospheric greenhouse shows why IGBP most urgently needs tough-minded management, able to distinguish the problems whose solution will lead quickly to better understanding from those that can be allowed to wait. This will not be an easy task. The intention is that there should be an international scientific committee to give shape to the IGBP, but that there should be no substantial central source of funds for supporting projects, which will have to look to national agencies for support. This is how the IGY functioned, but that success will be hard to repeat. One danger is that existing research programmes will simply be rechristened as components of IGBP without reference to the common objectives of the programme. Another is that, where extra funds can be found, the money will be spent on making many measurements that can be made with relative ease, not on the more difficult investigations on which understanding hangs. This is why the International Biological Programme, one of the successors to IGY (after the International Year of the Quiet Sun) was a relative failure. The new management will have to avoid this pitfall by sheer intellectual persuasion, which is a tall order.

Where will it all lead? The plan is that IGBP should produce within a decade a sufficient basis for making predictions in critical areas valid for a century or so in advance, which is a clarifying objective. But there are many fields, that of earthquake and volcano prediction for example, in which this ambition is unlikely to be satisfied, for which reason IGBP is also properly conceived of as an open-ended programme. One practical danger is that the politicians to whom would-be participants in IGBP must now turn will be demanding, long before a decade is up, to know the results and the implications for public policy. But some of the more important implications are already plain. The atmospheric greenhouse will, for example, require international conventions to restrain the emission of gases absorbing in the infrared, the negotiation of which is even more ambitious than the goals of the IGBP. Is the hidden agenda the hope that international collaboration on the scientific components of the global problems show the politicians show where to begin? That would be a great benefit in its own right. □



Strategic arms meeting

The need for general discussion on control agreements.

As the year wears on, the prospect fades that there may be a high-level meeting between the Soviet Union and the United States before 1987 is here, which will be a great misfortune. Virtually the only tangible agreement at Geneva a year ago was that President Reagan and Mr Gorbachov would meet again about now in Washington. Mr Reagan seems to want a meeting, even a meeting at which nothing is decided, but also seems prepared to let the matter slide. Mr Gorbachov, on the other hand, seems to take the view that a meeting not preceded by an agreement on what will be agreed is not worth having. Because Mr Gorbachov has spent much of the time since the Geneva summit producing a string of proposals for agreements on the limitation of strategic arms, without much response from the United States, his position on a second summit must certainly command sympathy. But that does not necessarily imply that it is correct.

Even a meeting without an agreement would be more than merely symbolic. Although it is mutually agreed that the general area of discussion is that of strategic arms control, neither side has a clear idea of what should be the objectives of serious negotiations in the changed circumstances following a decade-long dearth of treaty-signing ceremonies. Not that Soviet ambitions are a secret. Mr Gorbachov wants a test-ban (and is keeping unilaterally to a moratorium on nuclear tests), the abandonment of the Strategic Defense Initiative (but more recently he has said that laboratory research would be tolerable) and has put forward a variety of plans for reducing the numbers of strategic missiles below the limits specified by the Salt II treaty. Mr Reagan appears not to want anything passionately (although the test-ban and the abandonment of SDI are beyond the pale). The negotiations at Geneva seem nevertheless to have reached the point at which the two sides are not far apart in talking of reducing the numbers of strategic warheads by roughly 25 per cent.

So why not sign such an agreement and call a celebration? Because such an agreement would mean nothing without a framework of more general understanding about the problems occasioned by the existence of huge stocks of nuclear weapons. Put simply, a ban on nuclear tests would not, by itself, reduce the chance of mutual annihilation; there would still be plenty of workable weapons in the stockpiles. Nor would an agreement to work within a more restricted formula like Salt II, at least if the reductions were only of the order of 25 per cent. Moreover, there is also something in the arguments of those, called hawks, who say that substantial reductions of strategic arms would be dangerous by weakening the feat that attack would bring swift retaliation.

To the extent that SDI could upset the present balance, giving one side the fear that the other could launch an attack with impunity, the case for regulating that can of worms by mutual agreement is far stronger, perhaps on the basis of a reaffirmation of the Anti-Ballistic Missile Treaty pending renegotiation when the research phase of the programme is complete and the Soviet Union has been made privy to the outcome (as President Reagan has promised). But long before then, the technological basis of nuclear warfare will probably have changed yet again.

That is why strategic arms control agreements between the United States and the Soviet Union are not ends in themselves, but means to other ends. Both governments have a mutual need to get somewhere by 1995, when the Non-Proliferation Treaty has to be reaffirmed by its signatories, but that is still a distant agenda. The more immediate consideration is that arms control agreements could pave the way to a better political relationship between the two superpowers (as well as allowing third parties to sleep more easily at night). So why not put the cart before the

horse, and arrange a summit meeting to talk about the ways in which the superpowers would soften their attitudes towards each other in the event that some kind of agreement is eventually reached? Then the whole process would be easier. □

Wall Street jitters

Last week's slump does not presage another Great Crash, but the need to get rid of US deficits.

THOSE who knowingly skate on thin ice are a nervous lot. That is the simplest and most comforting explanation of the dramatic collapse of the New York stock markets at the end of last week. Last Thursday, the average value of stocks fell by 4.6 per cent. For the week as a whole, the fall amounted to 7.4 per cent (by the yardstick of the index known as the Dow Jones industrial average). Historically minded stockbrokers' men have been sickened to discover that, apart from a bleak day in May, 1962, there had not been a day when US stock prices declined as fast as last Thursday since 28 October, 1929, part of the prelude to the Great Crash that preceded the Great Depression.

The same people have also been ready with explanations of why the sombre parallel is not applicable. Now, they say, the markets are computerized to such a degree that, without human intervention, huge quantities of stock are off-loaded whenever there are a few pennies to be made by selling now and using the proceeds to make future purchases of the same, or similar, bits of paper. But that could account for the volume of sales (Thursday's temporary record was beaten on Friday) but not for the slump in stock prices. Another popular explanation is that the markets had been fed with rumours of impending economic indicators suggesting that both the rate of inflation and of economic growth in the United States were increasing, which would imply no further reduction of interest rates; the explanation would have been more convincing if the markets had turned round on Friday, when the rumours were proved false.

The truth is to be found elsewhere, well away from Wall Street, the financial centre in New York. By any standards, this is a curious time in the economic history of the United States. Both the federal deficit (the difference between the government's income and spending) and the country's trade deficit (the cost of imports less that of exports) are higher than they have ever been. The first gap is bridged by borrowing from US citizens the money that would, in other circumstances and under different administrations, be raised by increased taxes, the second by the flow of money from abroad, previously from the oil-producing states but now chiefly from West Germany and Japan. The upshot is that US citizens are being given increasing claims on future government revenues (which people will need if they are to enjoy their retirement in the conventional style) while increasing chunks of US commercial and industrial assets are owned from overseas.

On its present scale, this symbiotic relationship could continue indefinitely, provided that the US economy remains productive, but it is hard to see how the system can remain stable if the deficits persist, especially as the signs multiply that deflation continues, making more plausible every day the assumption that the Congress will shrink from taking the Gramm-Rudman axe to the budget deficit for the financial year beginning at the end of the month. Is it even possible that the still-hidden trigger for Wall Street's decline last week was merely the return of the Congress from its brief vacation reminded Wall Street that nothing much would change in an election year?

None of this implies that the United States (or the rest of us) are in 1929 again; the stock markets will no doubt partly recover the losses they have sustained. What this warning crack of the thin ice means is that it is not possible to postpone indefinitely the inevitable adjustment there will have to be between present expectations of prosperity in the United States and the lesser capacity of the economic system to satisfy them. □

Environmental pollution

UK denies responsibility for Scandinavian acid rain

THE government of Norway, downwind of the high chimneys through which Britain discharges sulphur dioxide and other gases from its power stations, is not in the least impressed by the British decision last week to fit desulphurization plants to three of the offending stations. Announced in London while British Prime Minister Mrs Margaret Thatcher was in Norway visiting her opposite number, Mrs Gro Harlem Brundtland, the decision was described by a Norwegian environment ministry spokesman as a "slap in the face" for Norway, which had been expecting a much greater gesture.

Yet according to Lord Marshall, chairman of Britain's principal emitter, the Central Electricity Generating Board (CEGB), the British decision was based on "new research" on acid lakes and streams in Scandinavia and there is at last a "reasonably sound scientific basis" for CEGB actions. These aim for a reduction of about 10 per cent in CEGB sulphur emissions by the year 2000. Scientists led by the Swedish Environmental Protection Board say 90 per cent is necessary. The CEGB plans to achieve such cuts, but only by 2020, when existing coal-fired plants will have gone, to be replaced by new low-sulphur plants or nuclear power stations. This will be too late, say the Scandinavians, who claim for example that of 33,000 km² of southern Norway now affected by acid rain some 13,000 km², seven major salmon fisheries are now devoid of fish, and 2,500–6,000 Swedish lakes are also affected.

The research of interest to the CEGB includes some of the results from the joint research group of the Royal Society, the Royal Swedish Academy of Sciences, and the Norwegian Academy of Science and Letters (funded by the board and by British Coal), whose first report is due next year, and international work on sulphur dioxide transport. But the Scandinavian view is that the British generating board is making its "small" move towards desulphurization only because the new findings can be interpreted to reduce and limit the board's culpability. Among these findings, improved atmospheric models have halved the degree to which acid inputs in Scandinavia can be traced to British sources, and new soil measurements have reduced estimates of the likely improvements to be gained from any immediate reduction in sulphur deposition. Environmentalists and some Scandinavian experts, however, dispute the board's interpretation of the data.

Of the two main sets of results, the transport figures may prove the least controversial. The directly attributable British contribution to acid deposition in Norway as a whole is only 8.5 per cent of total depositions, less than Norway's own contribution, says the board. Anton Eliassen of the Norwegian Meteorological Institute in Oslo agrees. Eliassen himself made a major contribution to the study, which is part of the United Nations Economic Commission for Europe pollution monitoring programme (EMEP). The fall in the British contribution (30 per cent was the consensus a few years ago) is the result of an improved model using a much-reduced estimate of the dry deposition rates of sulphur dioxide on sub-freezing surfaces and snow, says Eliassen. These have been measured to be four times lower in mid-Norway in mid-winter than previously assumed. The rate of conversion of sulphur dioxide to sulphate is also now known to be halved in mid-winter, and five times the minimum in mid summer, and these effects have been included in the new transport model. With a winter peak in sulphur dioxide production, the result is that sulphur dioxide is transported on average much farther before deposition than earlier models predicted. But the resulting Norway-wide average for British depositions on the country of 8.5 per cent of the total obscures the fact, says Eliassen, that in the most sensitive, soft-water region of south-western Norway Britain contributes 16 per cent, compared to a local contribution of just 3 per cent.

Forty-five per cent of the sulphur de-

positions in the region are 'unattributable'; that is, they come from parcels of air which have been over the Atlantic for more than four days, beyond the limit of the EMEP model projections. What part of this does Britain contribute? Environmentalists suggest as much as half. But there are other large emitters in Europe, and even America, that must contribute to the sulphur dioxide over the Atlantic, and possible biological sources in the oceans. The new EMEP model allows for external inputs into the European area, however, and only requires some 14 per cent of additional sulphur. (No more than 10 per cent of this is likely to come from the USA, according to Eliassen.) The rest, then, must be recirculated European emissions, and a considerable fraction of that is likely to be British. Nevertheless it remained possible for Mrs Thatcher to claim that the proven British impact on Norway (as a whole) was small.

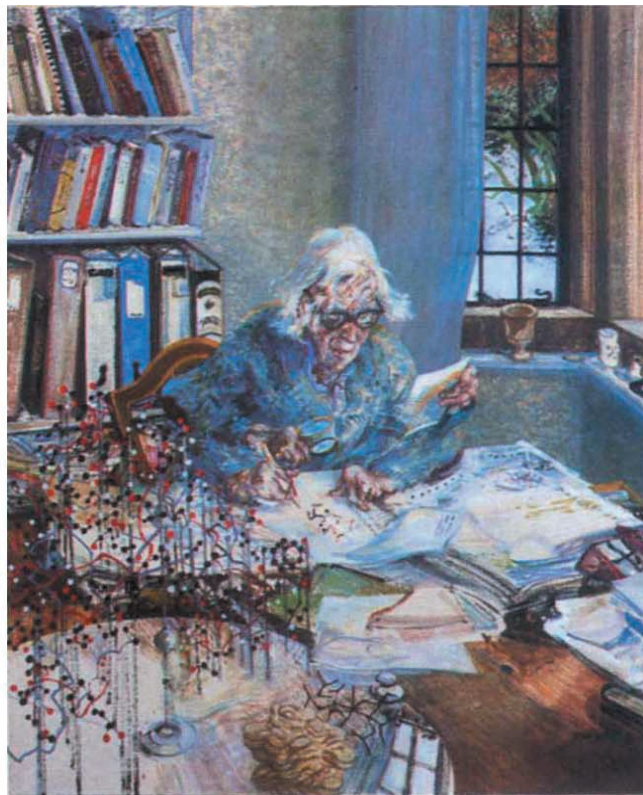
The second set of results to please the British generating board concern the quantities of sulphur that already exist in Scandinavian soils, whether or not it came from anthropogenic sources, and measurements of the effect of the deacidification of rain over a trial catchment area for 18 months. According to Peter Chester, chief environmental scientist of the board, in one Norwegian catchment (Risdalsheia) the reservoir of soil sulphur exceeded the amount of water soluble sulphate 100-fold, and in another (Gardsjon) 20-fold. The chemical nature of this sulphur is not certain, says Chester, but if it is mobilized — and there is evidence, he claims, that it can be mobilized by nitric acid flushes from biological decay after forest clearance — then it represents a sulphate reservoir amounting to decades of sulphur deposition at present rates. So, Chester argues, there is no point in reducing sulphur emissions rapidly, as this reservoir will continue to have its effect for years to come.

Robert Walgate

Prime Ministers Brundtland (Norway) and Thatcher (Britain) exchanging a few acid words?



From Newton to Rutherford: British scientists in oils



(Left) Edward Jenner (1749–1823) painted in 1802 by James Northcote. If anyone were now to try Jenner's famous experiment of 1796 they would probably find themselves in jail. He inoculated a boy with cowpox and then later with smallpox — without the latter causing illness. Vaccination spread throughout Europe at great speed and Jenner found himself a hero: he was even able to obtain the release of English prisoners of war by a direct appeal to Napoleon. (Right) Dorothy Hodgkin painted by Maggi Hambling (1985). Some of the earliest electronic computers were put to use by Professor Hodgkin to help extract molecular structures from X-ray diffraction data. In 1949, she obtained the structure of penicillin, and in the 1950s vitamin B₁₂. In 1964 she was awarded the Nobel prize.

Opening this week at the Science Museum in London is a special exhibition "Apples to Atoms: Portraits drawn from the collections of the National Portrait Gallery". Forty-five oil paintings and drawings of distinguished British scientists from the early seventeenth century to the present day will be on display. The exhibition then moves on to Norwich, Grasmere, and Coalbrookdale. □

US budget

Boom time for health research

Washington

WHATEVER the budgetary perils other agencies may face in the Gramm–Rudman deficit-reduction era, the National Institutes of Health, Congress's perennial favourites, seem likely to come out on top of the pile once again this year. Both the Senate and the House of Representatives have voted to provide the institutes with over \$6,100 million for fiscal year 1987, which starts on 1 October, \$1,000 million more than the administration requested. The provision of extra funds to combat AIDS (acquired immune deficiency syndrome) figured prominently in debates in both houses. The increase over this year's level would be more than 15 per cent at a time when many other government agencies are facing substantial cuts.

The amount could in principle still be altered if Congress is forced to enact "sequestrations" to agency budgets this

year, as it did last; but increasingly, Congressional staff seem to think it likely that a way will be found to avoid making the unpopular cuts, especially as a key provision of the Gramm–Rudman law has been found unconstitutional by the Supreme court and Congressional elections are due in November.

The House recommendation for the institutes was \$6,169 million; the Senate eventually settled on \$6,117 million, after a debate in which \$47 million was added to a committee recommendation in order to support satellite clinical trials for AIDS patients. The intention is that most or all AIDS and AIDS-related complex patients should be able to participate in a clinical trial of one of the experimental therapies such as azidothymidine. The \$47 million would also increase the number of AIDS treatment and evaluation units run by the National Institute of Allergy and Infectious Diseases from 14 to 19. The

total sum to be provided for the disease next year now looks likely to exceed \$400 million, almost 80 per cent more than last year.

Congress has also again made plain its displeasure with the administration's declared plan to reduce to 5,100 the number of new and competing extramural research grants awarded by the institutes; the Senate decided the figure should be not less than 6,175 grants, and the House 6,200.

Remaining differences will be resolved in conference. Last year an administration bid to circumvent Congress's directives on new grants by "multi-year" funding was challenged on legal grounds, so Congress is likely to get its way. **Tim Beardsley**

● **No change in party.** HEINZ Riesenhuber, Minister in charge of the West German Federal Ministry of Research and Technology is, of course, a Christian Democrat, not a Social Democrat as written in "Industry's subsidy attacked" (*Nature* 323, 4; 1986). Our apologies for this error, introduced during typesetting.

Tropical rain forests

Ecologists unite for diversity

Washington

A group of senior academic ecologists calling itself "Club of Earth" plans this week to issue a declaration stating that the loss of biological diversity caused in large part by the rapid destruction of tropical rain forests could constitute a threat to human civilization "second only to thermonuclear war". The group, which includes Edward O. Wilson of Harvard University, G. Evelyn Hutchinson of Yale and Paul Ehrlich of Stanford, foresees within the next few decades the extinction of a quarter of all animal and plant species on Earth unless decision makers implement policies that combine economic interests with the preservation of diversity.

The group, which will probably be restricted to 10 members, intends to make its first declaration at the start of a three-day symposium on "bioDiversity" to be held this week by the National Academy of Sciences and the Smithsonian Institution in Washington D.C. Club of Earth will then make occasional informed statements on the state of biological diversity for the public and for political leaders. The group believes ecologists have failed to make an adequate impact on public policy and will urge that they be routinely consulted on planning decisions.

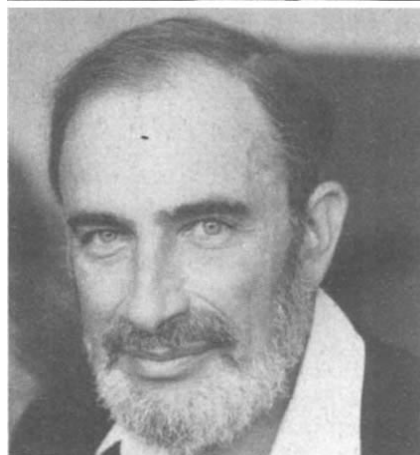
According to Wilson, membership of the new group has been restricted to distinguished scientists who are members of the National Academy of Sciences in order to ensure that it "cannot be ignored". Other members may include Jared Diamond, Peter Raven and Thomas Eisner. Wilson says the group's declarations, although political, will be "chaste and sparing".

Chaste and sparing would scarcely describe Paul Ehrlich's past diatribes against environmental mismanagement, but the data assembled by Wilson to illustrate the rate of loss of species are compelling. Wilson estimates there may be between 5 and 30 million species on earth, although only 1.7 million are described. Tropical rain forests contain over half of the total, but are being destroyed at the rate of 5.9 million hectares (more than the area of Costa Rica) per year, equivalent to 0.7 per cent of total cover. If this rate is maintained, all tropical rain forest will be clear-cut or seriously disturbed by A.D. 2135.

Wilson argues for a major effort by biologists to assess species diversity worldwide; he says the project is economically feasible and, unlike recent proposals to sequence the human genome (see *Nature* 322, 397), urgent because if it is not done now the species will have become extinct. He believes the case for slowing the rate of extinctions is not merely aesthetic; many medicines and foods may be developed

from novel species, and their germ plasm may yield valuable new hybrids.

Ehrlich says the Club of Earth has been formed in part because some biologists



Edward O. Wilson (top) and Paul Ehrlich (bottom) will help found Club of Earth.

were "very disturbed" by a National Research Council study of human population trends published early this year (Population growth and economic development; policy questions. National Research Council, 1986) which concluded that population growth in developing coun-

tries might in some circumstances not be a bad thing. Ehrlich says the study was "full of horrendous errors" that came about because it was conducted entirely by social scientists and economists and included no input from ecologists and evolutionists.

This week's symposium is plainly intended as a media event. It has been designed to explain biological diversity and its importance to the public and government, according to its organizers, and it results from growing concern about the issue at the Academy, where it is felt it has failed to receive the attention it deserves. Speakers representing all interests will be present, including the World Bank, currently under criticism for allegedly supporting environmentally unsound development projects, and from the US government's Agency for International Development (AID).

The maintenance of biological diversity has been slowly gaining prominence as a public issue in recent years. The National Wildlife Federation has published proposals to use natural resource conservation considerations, including biological diversity, as a *quid pro quo* for international debt "rollovers" and "forgiveness". Following Congressional directives made in 1983, AID now has a published strategy for encouraging the support of biological diversity and says it already encourages aid recipients to protect their biological resources, for example by developing conservation areas. Critics contend, however, that specific action so far has been slight.

Legislation now awaiting final Senate approval would oblige AID to spend \$10 million per year on new projects designed to encourage biological diversity in developing countries. Agreement between the House and Senate on the bill seems likely, probably with the \$10 million phased in over several years) but a presidential veto is possible; although AID says it is planning to take greater account of diversity considerations in its future projects, the administration is opposed in principle to the provision of earmarked funds.

Tim Beardsley

Japan finally says yes to Star Wars proposals

Tokyo

JAPAN has finally announced its intention to participate in the research programme of the US Strategic Defense Initiative (SDI). In making the announcement last week, Chief Secretary Masaharu Gotoda extolled the virtues of the programme, emphasizing its defensive nature and the potential benefits to Japan's high-technology industry. Opposition parties, however, roundly denounced the decision as running completely counter to a Diet resolution on the non-militarization of space and the war-renouncing provision of the constitution. But with the ruling party holding a stable majority, Japan's participation is assured.

Last week's decision followed 18 months of deliberations during which the Japanese government sent three missions to the US and held six cabinet level meetings to discuss the technological, strategic and legal implications of joining SDI. Technology will be transferred to the US under existing laws and Japan-US agreements, and the Ministry of International Trade and Industry will work out guidelines to ensure the "smooth" participation of Japan's industry — no Japanese company has ever concluded a consignment contract with the US Department of Defense, and there are some fears that SDI is an American plot to drain Japan of its latest high technology.

David Swinbanks

Indian science

Visa row threatens conferences

New Delhi

INDIA is unlikely to act as host to international science conferences in future because of a tiff between the International Committee of Scientific Unions (ICSU) and its affiliate, the Indian National Science Academy (INSA), over the unconditional right of entry of scientists wishing to attend the conferences. The question has cropped up because of allegations that South African scientists have had difficulty in obtaining visas to attend ICSU-sponsored meetings in India.

The Indian government, whose anti-apartheid policy is well known, does not ban entry of South African scientists. But visas are issued only to those who declare opposition to apartheid in writing, which many South African scientists do not want to do for fear of reprisals at home. ICSU wants this condition lifted as it violates the charter of "complete freedom of movement of scientists" to which INSA subscribes. In a letter to INSA, it threatened to withdraw support and recognition for international conferences in India if blanket entry permission was not provided irrespective of nationality.

INSA, which is partly funded by the government, is not in a position to challenge the government's policy of conditional entry to South African scientists and INSA president, Professor C.N.R. Rao says the Indian policy is justified. He says that while ICSU is asking India to abide by its charter on freedom of movement, it is not asking South Africa to conform to its rules banning racial discrimination. ICSU has never imposed any sanctions against the South African science community although its black scientists do not enjoy the same dignity and respect as the whites. Rao, who is also chairman of the International Union of Pure and Applied Chemistry (IUPAC), twice turned down invitations from the South African Academy of Sciences because the invitations said he would be treated as an "honorary white", which he believes is a clear admission of apartheid in science. INSA alleges that ICSU has singled out India while several other nations continue to deny visas to scientists for various reasons.

Despite restrictions, South African scientists have been attending conferences in India. The conference of the International Astronomical Union (IAU) last year was attended by a team from South Africa and IAU's vice president himself was a South African. But the special treatment meted out to South African delegates is a topic raised in every international meet. Some years ago, a conference on the mossbauer effect was postponed at the last moment following a protest from South African scientists whose visas were not

cleared. The issue came up again at the eleventh conference of the International Sociological Association (ISA) held recently in New Delhi. The British sociologist and ISA president Mrs Margaret Archer, disclosing how a South African delegate faced 'serious' problems in getting a visa, said she would have favoured shifting the venue if there had been total opposition to South African participation. Local organisers whose biggest headache is obtaining government clearance for South African delegates say their responsibility ends with the submission of their names to the Foreign Office.

The result of ICSU-INSA row is that India may not host any ICSU-supported conferences in the future. INSA has already advised scientific institutions in the country not to seek any support from ICSU or its member unions for holding meetings. According to Rao, India can find enough resources on its own to organise small international conferences. One other repu-

cussion is that some of the proposed international meetings in India are in jeopardy. Among them are the colloquium of the International Mathematical Union (1988), Conference on General Relativity (1989) sponsored by the International Union of General Relativity and Gravitation, and Biophysics Congress (1990) sponsored by the International Union of Pure and Applied Biology. If these conferences are held, they will be without ICSU support, says Rao. Also in doubt is the participation of eight South African eye specialists in a conference in December this year by the National Society for Prevention of Blindness, a private body. ICSU's retaliatory move against India, spearheaded by the standing committee for freedom of movement of scientists with generous backing from some western scientists, is seen by INSA as nothing but politics, and Indian politicians just back from the non-aligned meeting at Harare, where they mounted a vigorous attack against apartheid, are in no mood to change their government's declared policy in order to accommodate ICSU's demands.

K.S. Jayaraman

Appeal launched for Soviet biologist

DR Alex Goldfarb, the son of David Goldfarb, the Soviet Jewish molecular biologist implicated in the case of the United States journalist and alleged spy, Mr Nick Daniloff, has called for a campaign of telegrams from western scientists on his father's behalf.

David Goldfarb, a diabetic, is seriously ill in a Moscow hospital, awaiting amputation of his remaining leg for gangrene. Developments of the Daniloff case last weekend have led his son to fear that, without strong international protests, the operation might be fatally delayed or withheld.

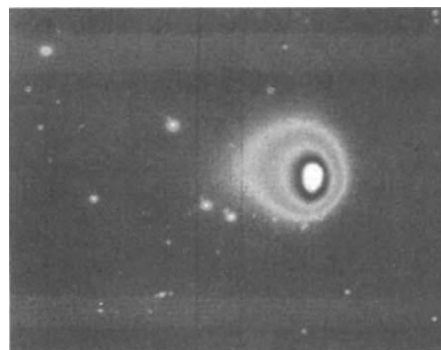
David Goldfarb is a long-time acquaintance of Daniloff who regularly supplied him with genetically-engineered insulin from the United States. Recently, he revealed that in the Spring of 1984 when he was preparing to leave the Soviet Union, he was approached by the KGB to plant classified material on Daniloff. When he refused, his exit visa was withdrawn and he himself was accused of trying to remove from the Soviet Union material of national importance — certain bacterial strains used in his research — although these had originally been obtained in the west (see *Nature* 301, 104, 1984).

Last Saturday, Gennadi Gerasimov, a Soviet government press spokesman, denied this story and said that David Goldfarb had admitted to a TASS reporter that it was untrue. But Alex Goldfarb alleges that it is Gerasimov who is lying and asks his father's colleagues to demand that an international press team be allowed to visit his father. He also calls for a campaign to

get his father transferred to a hospital in the United States, or failing that, for visas to be granted to the two US doctors, Kenneth Prager of Columbia Medical School and Glen Geelhoed, of George Washington University, who have agreed to attend him in Moscow.

Vera Rich

Comet coming



Pasadena

A new comet, discovered last month by Christine Wilson, a graduate student at Cal Tech, may put on quite a show in April 1987, when its orbit will bring it within about 110 million miles from the Sun. Comet Wilson was discovered while using the 48-inch Schmidt Telescope at the Palomar Observatory for the Palomar sky survey. Estimates suggest its brightness will reach 3.5, about the same as Halley's Comet. Initial observations suggest Comet Wilson is a long period comet, with an orbital span in the millions of years. It will be visible only from the southern hemisphere.

Joseph Palca

Uranium export

Australia puts idealism aside

Canberra

WITH the decision to lift the ban on uranium sales to France announced in the budget, uranium is once again a hot issue in Australia. The decision has infuriated the left wing of the Labor party. As they heard the decision announced three left-wing back benchers made an unprecedented protest by noisily walking out of the budget session. Outside parliament the decision triggered the resignation of one of Prime Minister Hawke's senior advisors and a vigil over a consignment of uranium in Darwin which protestors believe is destined for France. The Labor left have sworn to do everything they can to overturn the decision.

The ban was put in place in 1984 to protest at France's nuclear weapons test in

angry because the budget pronouncement came only weeks after Labor's annual national conference, where the policy of banning sales to France was reaffirmed.

The ban was one of the last victories for the anti-uranium faction which was at its strongest while Labor was in opposition. But uranium has not always been synonymous with evil as far as the Labor party is concerned. The Whitlam government of the early 70s was enthusiastic about its

potential as an export earner. The period even saw a visit by a government minister to Iran to try to sell uranium to the Shah. Since 1977 a series of compromises for the sake of miner's jobs and political expediency have reduced Labor's anti-uranium policy to tatters. One compromise saw the opening of the Roxby Downs mine in South Australia, potentially the world's largest. Today Australia exports \$300 million worth of uranium per year. As Treasurer Paul Keating put it "when the Labor Party decided to mine Roxby Downs and let Ranger export, it decided not to have an anti-uranium policy".

Charles Morgan

European space programme

Germany plans a space plane

Hamburg

FIRST Hermes, then HOTOL and now "Sänger". France and Great Britain have been joined by West Germany in proposing a totally new launch vehicle for the future space race with the United States and the Soviet Union.

Sänger, named after the German aerospace engineer Eugen Sänger, who died in 1964, is the result of joint work by Messerschmidt-Bölkow-Blohm and the German Aerospace Research Establishment and is based on ideas Sänger had back in the 1940s. The spacecraft is seen as lying in between Hermes, a small shuttle to be launched on Arian 5 (an upgraded version of the European Space Agency's rocket) and HOTOL, which requires the development of a new engine, working as a jet up to 30 km altitude and then turning into a rocket. The idea behind Sänger, considered by the United States before they fixed on the space shuttle, is to use a large (50m, 400 ton) air-breathing, delta-winged hypersonic aircraft to carry a smaller winged orbital vehicle (length

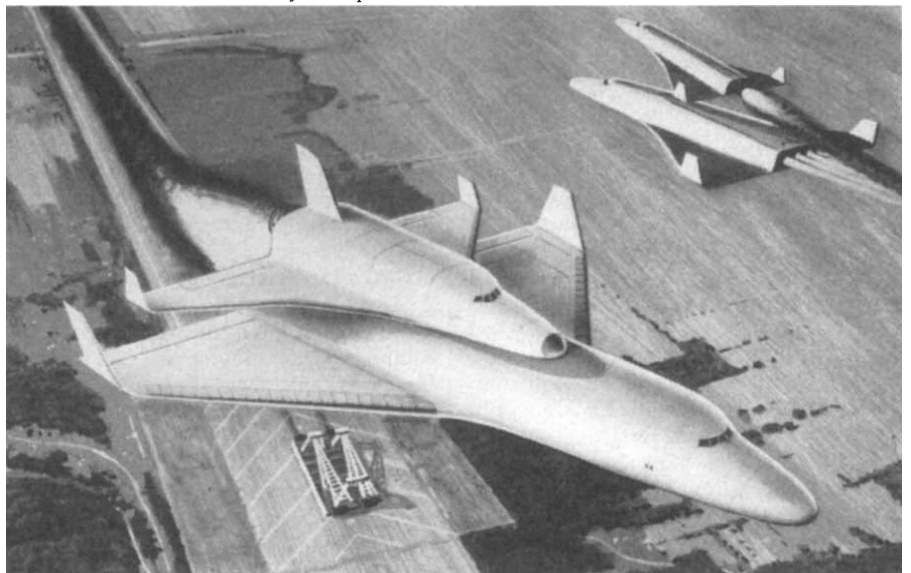
25m, weight 50 tons of which 35 tons would be fuel) up to an altitude of 30 km and a speed of Mach 6. There the orbital vehicle's cryogenic engine would ignite and carry it into orbit; later it would land, like its mother plane, at a conventional airport.

The orbital vehicle would carry a crew of two, along with ten passengers and a cargo of up to four tons. Its designers claim launch costs would be a fifth of those calculated for Ariane 5. And the project would give Europe a chance to keep abreast with US plans for an "Orient Express" transatmospheric vehicle, for which more than 500 million dollars have been appropriated. Sänger's Mach 6 air-breathing plane could be converted to a passenger version capable of carrying 200 passengers more than 20,000 km.

West Germany has asked the European Space Agency to study Sänger in parallel with HOTOL, its direct competitor. It is seen as a logical follow-on to Hermes which could fly by 1995; Sänger will not be ready before 2010.

Jürgen Neffe

The German shuttle hitches a lift into space.



the South Pacific. The justification now given for revoking the ban is that it has had no effect. France continues to test in the South Pacific, they have been able to buy uranium more cheaply elsewhere, and Australia desperately needs the money. According to the Treasurer, Paul Keating, abandoning the ban will bring in \$66 million this financial year, helping right the country's woeful balance of trade.

Prime Minister Hawke's answer to the assertion by Labor's left wing that he must be bound by Labor party policy is that in times of economic crisis the government has the right to overturn party policy. Hawke's position was upheld by a 74-42 vote of the Labor parliamentary caucus. In an attempt to embarrass the government the Australian Democrats, who are represented in the Senate, have moved an amendment drafted directly from official Labor party policy banning uranium sales to France. If the amendment comes to a vote government members will have to join the opposition to vote for their own party's policy! The left is all the more

New materials

Japan set to come out winner

Washington

JAPAN, not the United States, will capture the large new market for advanced ceramics and composites according to a new study* from the US Office of Technology Assessment (OTA). The study pulls no punches in blaming US industry for failing to match Japan's well-coordinated effort to develop and commercialize advanced materials technologies.

Much of the initial research that demonstrated the feasibility of new high-performance materials was done in the United States but foreign industry and industry-government teams, especially in Japan, have "far more aggressive programmes to commercialize near-term applications" says OTA.

Advanced ceramics, such as those based on silicon carbide/nitride or zirconium oxide, with their inherent advantages of lightness, strength and temperature resistance, have the potential to replace metals in many engineering applications. According to Congress's research office, the potential will be realized only when the design of the materials and the structures they are used in become integrated, as yet not possible on a large scale.

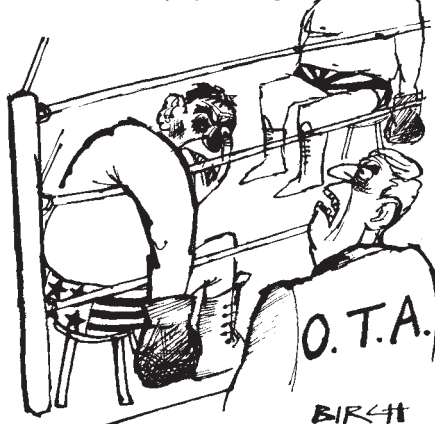
The federal government will spend \$60 million this year on research in this field. But industry has been slow to follow up on even non-military federal research, according to OTA, and industrial investment must be encouraged. Commercialization of military research, accounting for about one half of the federal outlay, is further inhibited by restrictions on publication of research results and on exports.

The size of the US market for ceramics

in the year 2,000 is estimated at between \$1,000 million and \$5,000 million. By way of comparison, the world market in 1983 was only \$250 million. The main applications will be in bearings, heat exchangers, and automobile engine components; in the longer term they may be used in advanced engines, if some major technical

How many more times?

Come out fighting!



obstacles are overcome. But OTA's verdict is that US industry considers the risks to be too high to conduct the necessary long-term research, and that more federal efforts are necessary. The priorities should include materials processing, reliability and durability in different chemical and physical environments, and tribology, testing and joining.

Tim Beardsley

* New structural materials technologies: opportunities for use of advanced ceramics and composites. Office of Technology Assessment, 1986.

Nuclear power

Accident close to the border

Hamburg

AN operator error at the just-completed French nuclear power plant at Cattenom has provoked a storm of protest in Luxembourg and West Germany. During a test run of a part of the plant, located near the border with Luxembourg and Saarland, more than 400,000 tonnes of cooling water swamped the cellars, seeped into the soil and flowed under ground below the village of Cattenom back to the Mosel river.

The water contained little or no fission products. But critics, took offence at the behaviour of the plant's operator, Electricité de France (EdF), which, a day and a half after the accident, said that only 8 tonnes of water were lost.

While Jo Leinen (Social Democrat), environment minister of the Saarland, reproached France for "making all the mistakes that could be made", *Le Monde* accused the Germans of reacting hysterically. Leinen has already criticised the design of the Cattenom plant saying that the quality of its steel and concrete is not high enough and that it lacks a steel dome to protect the reactors from an aeroplane crash, although there is much air traffic in the area.

The Governments of the Saarland and of ten towns and local districts, along with Luxembourg, have now opened proceedings at the Administrative Court in Strasbourg, to try to defer the start up. At a hearing on September 6 the plaintiffs' hopes were raised when the French state commissioner Antoine Mendras, accepted two of their seven objections: that the EdF built four 1,300 megawatt blocks, although it only had the permission to build two 900 and two 1,300 megawatt blocks; and that it did not inform the EG commission of its plans to discharge radioactive fluids into the Mosel river, although obliged to do so according to article 37 of the Euratom agreement. But on the 8th of September the court ruled not to cancel the permission for operation. All parties represented in the Saarland parliament protested against the judgement.

The German Federal Government is now placed in a difficult situation. Chancellor Helmut Kohl and his environment minister Walter Wallmann (both Christian Democrats) would like to polish up their images by making the French government improve safety standards — French plants are permitted to release five times more radioactivity than German ones. But Wallmann realizes from his July visit to Paris that the French do not appreciate criticism of their energy policies.

The Greens in the federal Government are now demanding Wallmann's resignation.

Claus P. Dechau

Resignation provokes criticism

Washington

WHATEVER the real extent of the blow to the X-ray laser programme at Lawrence Livermore laboratory caused by the resignation last week of Peter Hagelstein, the physicist credited with performing the basic theoretical calculations for the device, press attention could not have been less welcome to the administration.

The nuclear pumped X-ray laser is the most controversial item of the Strategic Defense Initiative (SDI), since its use would seem to require nuclear explosions in space. Next year's budget for SDI, of which the X-ray laser programme is a key part, has still to be decided by Congress but may be very much less than the \$5,300 million requested by President Reagan.

Hagelstein, who will take up a post as associate professor at Massachusetts Institute of Technology (MIT) in October, has not told the reasons for his departure, and it was speculated that he had become disen-

chanted with the military application of his work. The story of how Hagelstein left MIT, to take advantage of powerful equipment for investigating X-ray lasing at Livermore, has been told by William Broad of the *New York Times* in his book "Star Warriors". Broad wrote that Hagelstein originally wanted the X-ray laser to be used for medical research and that he felt he had his "arm twisted" into doing weapons research.

But according to Professor Richard Adler of MIT, associate head of the department of electrical engineering and computer science and a personal friend of Hagelstein, his decision to return to MIT was perhaps simply a reaction against the "mission oriented, driven" life in a defense research laboratory. Hagelstein will do work on computational physics in the department, probably concentrating on the problem of how to build a laboratory-scale X-ray laser.

Tim Beardsley

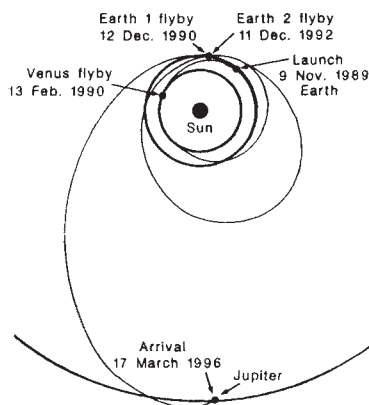
Space probes

Planetary tour for Galileo

Pasadena

THE fastest way to Jupiter is not via Venus, but for the long-delayed Galileo Jupiter probe and orbiter that may be the only way to make the trip. With a trajectory that evokes images of a complex billiard shot, the plan is now for Galileo to take a gravity boost from Venus and then two more from the Earth before heading out toward Jupiter. The multiple fly-bys will add more than three years to the mission but, project scientists at the Jet Propulsion Laboratory (JPL) all agree, it beats sitting on the ground.

Galileo was scheduled for launch aboard the space shuttle in May of this year. Had everything gone according to plan, a Centaur liquid-fuelled upper stage would have sent the spacecraft on a direct trajectory toward Jupiter. But the January Challenger accident, in addition to halting all launches, prompted a re-evaluation of shuttle performance characteristics. Even using a powerful Centaur upper stage, launch weight restrictions had mission planners considering an Earth gravity assist to change Galileo's velocity with respect to the Sun, a so-called delta VEGA trajectory. In June, however, the National Aeronautics and Space Administration (NASA) decided the safety risk of carrying the Centaur with its volatile fuel inside the shuttle was too high, forcing Galileo's



Playing planetary billiards en route to Jupiter.

managers to look elsewhere for an upper stage (see *Nature* 321, 800, 1986). By using the IUS solid fuel booster rocket already launched aboard the shuttle, coupled with a kick stage and expanded fuel tanks aboard the satellite, Galileo could make it to Jupiter using a delta VEGA trajectory. But that plan died when new safety margins for launch abort landings prompted NASA to lower cargo weight.

Prospects for the mission looked bleak in mid-July. Then Roger Diehl became involved. He had been responsible for designing Galileo's orbital tour of Jupiter's moons, taking a gravity boost from each moon to power a visit to the next. Diehl

substituted the Sun for Jupiter in the software used for the satellite tour, treating the planets as he had the moons of Jupiter. He discovered that using the counter-intuitive approach of launching toward Venus in late 1989, then returning to Earth, he could arrive back at the Earth at just about the right time to hook into a previously calculated delta VEGA trajectory proposed for a Titan launch in 1990. Now he knew there was an orbit that would not only provide the proper energy to reach Jupiter's orbit, but would also arrive when Jupiter was in the right place.

The new trajectory, christened VEEGA for Venus-Earth-Earth-gravity assist has its drawbacks. In addition to the extra travel time, new heat shielding will have to be added to the spacecraft for the trip close to the Sun. But the trajectory

has advantages as well. It can be achieved using the shuttle and the IUS upper stage without an additional kick stage, and it no longer requires a major mid-course burn to achieve the correct fly-by angle for the final pass of the Earth.

Project scientists are elated by the VEEGA trajectory. Now mission scientists at JPL must hope that NASA headquarters will agree that Galileo is important enough to launch aboard the shuttle once flights resume. Galileo's launch window is very close to one needed by Ulysses, the collaboration between NASA and the European Space Agency for a polar orbiting mission. Political pressures are great to launch Ulysses as soon as possible, and there is some doubt that both will make a 1989 launch date. Still, VEEGA has given Galileo scientists hope that their spacecraft may someday leave its earthbound hibernation for a more fulfilling role in orbit around Jupiter.

Joseph Palca

Biotechnology

Genetic engineering minus glamour

Washington

CHEMICAL industry gave a vote of confidence to the potential of genetic engineering to improve industrial processes last week with the formation of a new company, Celgene. A spin-off from the \$3,000 million Celanese Corporation it will be headed by former Monsanto chairman, Louis Fernandez.

The industrial chemicals business has always lacked the glamour of pharmaceuticals. No hepatitis vaccines or growth hormones here: the cream of the research crop will be agents that thicken toothpaste and ripen cheddar cheese. The products need not be pure, but they must be plentiful. And cheap; whereas a recombinant drug might sell for \$10,000 per gram, a gram of industrial enzyme usually costs less than \$1.

The market potential is enormous. According to the US Office of Technology Assessment, the US chemical industry as a whole will be buying \$14,000 million worth of recombinant products annually by the year 2000.

To achieve that expansion, existing industrial enzymes will be improved to enhance the substrate specificity, product control and processing economics and novel enzymes designed for new tasks. But so far only one recombinant product for industrial purposes has cleared the Food and Drug Administration: a heat-stable alpha-amylase made by CPC International. Approval took two years, and the enzyme is still ensnared in environmental assessments. "The expense and time it takes for regulatory approval just doesn't make sense for a product that sells for \$2 a pound," admits a manager at CPC's Mof-

fett Technical Center.

Indeed, cost is what has made the enzyme industry reluctant to embrace biotechnology with the fervour of the pharmaceuticals. Genencor's Jonathan MacQuitty points out that manufacturing costs comprise about 6 per cent of the sales price for pharmaceuticals, but as much as 60 per cent of the costs of industrial enzymes. The industry simply cannot afford expensive production techniques. While prices should fall as high technology becomes routine, many companies are adopting a "low-tech or no-tech" approach.

Walter Goldstein, who heads the biotechnology group at Miles Laboratories, has formulated an algorithm to determine the feasibility of making a bioindustrial product by a given means. He says more research on organisms and substrates is required to make biotechnology pay in the biocatalysis business. Industry prefers filamentous fungi and *Bacillus* bacteria to the cultures used in pharmaceutical production, and they are difficult to engineer, and will take time to perfect. Scale-up provides another problem: while drug doses are measured in milligrams; speciality chemicals come in trucks.

Even when the enzymes industry reaps the harvest of recombinant technology it is likely to remain reticent. Biocatalysts are often intimately related to customers' proprietary processes. To preserve confidentiality, companies like Celgene and Genecor favour partnerships and contract agreements to research on products for general distribution. It is likely, says MacQuitty, that the most spectacular enzymes will always remain "the most secret of secret things."

Karen Wright

Fluoride, immunity and tooth decay

SIR—Diesendorf¹ has raised fundamental questions about the supposed benefits of fluoridation in preventing tooth decay. May the debate continue.

As to why tooth decay in developed countries has declined, consider the following: the usual and first explanation for a marked fall in the incidence of any infectious disease is the acquisition of specific immunity to the causative organism by a significant proportion of the population at risk. Specific immunity may be acquired naturally or artificially (vaccination). Naturally acquired specific immunity may explain the decline in caries in developed countries. Tooth-brushing is now widespread in pre-school children and as many as 75 per cent of children may be brushing their teeth by the age of 18 months². Current techniques teach children to brush the gums gently as well as the teeth. The bristles of a tooth-brush together with the mild abrasives found in toothpaste make an ideal instrument for transferring and implanting antigenic material from around the teeth, that is, decay-causing bacteria, into the oral mucus membranes.

The mouth possesses an immune system, and murine oral mucosa, at least, contains both the effector and regulatory cells required for the local production of immunoglobulin³. Brushing the gums with a contaminated brush may therefore produce a massive antigenic challenge resulting in the production of SIgA immunoglobulins⁴. The immunoglobulins may then cross cell barriers into the oral fluids, or they may form an SIgA-mucin complex on the surfaces of teeth. In either location they may interfere with the ability of decay-causing bacteria to attach to teeth.

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SIR—Mark Diesendorf's account of "the mystery of declining tooth decay" ignores the study of the effects of fluoridation in Anglesey¹, which was conducted under strictly blind conditions. This study clearly demonstrated a reduced incidence of dental caries in children in fluoridated Anglesey, as compared with controls living in non-fluoridated Bangor and Caernarfon. A secular reduction in the incidence of caries was observed in both communities between 1974 and 1983, possibly related to the increased use of

fluoridated toothpastes, but in fluoridated Anglesey there was an additional reduction, particularly in the incidence of approximal attacks of molar and premolar teeth.

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Classical caries

SIR—Wyborny and Shannon¹ expose the lack of "classicism" in Coca-Cola. They also discuss the possible cariogenicity of various forms of this beverage. The "FASEB report"² noted that several investigators "found sucrose to be the most cariogenic substance among sugars and foods tested in animal experiments", but in other studies, glucose, fructose and other sugars were "almost as cariogenic as sucrose". Cariogenicity was related to adherence to the teeth by foods. A 10 per cent sucrose rinse was no more productive of low plaque pH values than were several other foods, including wheat flakes³.

Wyborny and Shannon speculate that either increases or decreases in dental caries may result from changes in Coca-Cola. This might be difficult to assess in view of the current decrease in dental caries attributed to water fluoridation and fluoride dentifrices⁴.

Classically speaking, Aristotle observed that "soft, sweet figs adhered to the teeth and produced damage".

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Prelude to space

SIR—May I correct your News item about space vehicles (*Nature* **322**, 98; 1986)?

I certainly never claimed to have "invented" the idea of air-breathing engines for space vehicles 40 years ago. The concept is much older.

I merely said that I had described a (nuclear-powered!) horizontal take-off air breather in the novel *Prelude to Space* in about 1948.

ARTHUR C. CLARKE

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Anadin not anodyne

SIR—In the editorial "Second chance for nuclear power?" (*Nature* **323**, 1; 1986) you likened the switching off of safety systems at Chernobyl to a person with angina attempting to hide the pain by swallowing aspirin. Far from it: swallowing aspirin is a good bet, at least for those patients with unstable angina^{1,2}.

J. M. RITTER

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Poe's universes

SIR—I read with interest and approval E. Harrison's article on Kelvin and the Olbers paradox (*Nature* **322**, 417; 1986). I was glad to see that Edgar Allan Poe and his masterpiece *Eureka* were once more rehabilitated in a scientific magazine. *Eureka* contains a diversity of ideas and visions that show that Poe was far ahead of his time.

I do not, however, agree with Harrison when he says that Poe means 'galaxy' when he writes 'universe', because this does not do justice to Poe, who was a careful writer. The words 'universe' and 'galaxy' are often used in *Eureka*, and they are not freely interchangeable.

The point is that Poe did have a vision that closely resembled a concept nowadays called "multiple", "parallel" or "simultaneous" universes in different dimensions (and therefore unknowable to each other). Harrison quotes only the first sentence, but only with the above explanation does the whole paragraph make sense:

Let me declare only that, as an individual, I myself feel impelled to fancy — without daring to call it more — that there *does* exist a *limitless* succession of Universes, more or less similar to that of which we have cognisance — to that of which *alone* we shall ever have cognisance — at the very least until the return of our own particular Universe into Unity. *If* such clusters of clusters exist, however — *and they do* — it is abundantly clear that, having had no part in our origin, they have no portion in our laws. They neither attract us, nor we them. Their material — their spirit is not ours — is not that which obtains in any part of our Universe. They could not impress our senses or our souls. Among them and us — considering all for the moment, collectively — there are no influences in common. Each exists, apart and independently, *in the bosom of its proper and particular God!*

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Helices with optical activity

A neat conformation of a neat prediction points to more problems in the interpretation of quantum mechanics. In what direction do we turn now?

THE complexities of quantum mechanics persist not merely at the cutting edge represented by particle physics, but in much more elementary manifestations. It is a curious state of affairs. Students are taught how to calculate the wave function of, say, the state of an electron, but many go on to spend their lives worrying about quantum chromodynamics, string theory and the like; yet quite simple questions remain unanswered. Why, for example, is it supposed that the square of the amplitude of a wave function represents the probability distribution of the electron? It is justifiable chiefly because it works. Now there is a rush of interest in a set of problems springing from another long-recognized ambiguity in the calculation of a wave function, that is whether the phase of the result has physical significance of any kind. One of the most recent developments appears to vindicate an important prediction (*Proc. Roy. Soc.* **45**, 1984) by Dr M.V. Berry at the University of Bristol.

The problem is usually well taught to students. The solution of, say, a Schrödinger wave-equation for an electron in a time-independent electrical potential is a function which is, in general, algebraically complex: the value of the function at any point is a complex number, with a real and an imaginary part. The obvious physical correlate of this result is the probability that the electron will turn up at that point, which is simply the square of the amplitude of the complex number, or the sum of the squares of its real and imaginary parts. In that representation of complex numbers by a point in the two-dimensional plane in which the real and imaginary parts are the displacements from the origin along two perpendicular axes, the amplitude is the length of the radius vector to the representative point. The phase, the angle whose tangent is the ratio of the displacement along the two axes, appears to have no physical significance.

All representations of quantum mechanics have the same property. Whether the states of systems are represented by functions (Schrödinger), vectors (Heisenberg) or elements in a formal algebra (Dirac), all these quantities may be multiplied by complex numbers (or functions) chosen arbitrarily except for the condition that their amplitude is always exactly unity without the calculation of the usual physical quantities being affected in any way. So is the phase entirely without physical

significance? And if so, why cannot quantum mechanics be constructed so as to dispense with it?

People agreed on this until about twenty years ago, extrapolating from the Aharonov-Bohm effect. While the phase of a wave function representing the state of some system may be irrelevant in the calculation of measurable quantities such as the total energy, the place to look for the effects attributable to phase is among interference phenomena, for which purpose only changes of phase can matter. There are circumstances in which the phase of, say, an electron wave-function, however arbitrary or irrelevant, may be made to vary systematically. Magnetic fields (which may deflect electrons while doing no work on them) may do the trick; there have recently been at least two demonstrations of interference between the two halves of a split electron beam whose phases have been changed differently by magnetic fields.

Berry's argument is a generalization of the Aharonov-Bohm effect. The properties of a quantum state may potentially be determined by many parameters; an externally applied magnetic field is merely one. Each parameter will appear in the Hamiltonian representing the energy of the system as a whole, to each value of each of which corresponds a different set of stationary states. It is possible to imagine that one of these parameters is altered systematically, but so slowly that a stationary state becomes another (adiabatic is the word), and in a way that returns it to its starting-point. Physically, the system will then be back where it started. Will the phase of the wave function have been changed? Berry says yes, in general.

Recent intriguing evidence bears him out. The physical system is the simplest possible, the polarized photon, characterized by just two dynamical variables; the wave vector (which must lie in the direction of propagation) and the sense of polarization (which may be left-handed or right-handed, viewed along the direction of propagation). Raymond Y. Chiao from the University of California at Berkeley and Yong-Shi Wu from the University of Utah now point out (*Phys. Rev. Lett.* **57**, 933; 1986) that, for circularly polarized photons in, say, an optical fibre, the propagation vector, is simply the direction of travel, is determined by the shape of the track followed by the fibre, and thus is a parameter in Berry's sense. If the fibre is

not too sharply kinked, photons will remain polarized with sense unchanged.

What happens to the phase? It depends on the contortions of the propagation vector as the photon travels along the fibre. Chiao and Wu infer from Berry's work that two fibres of the same optical path length wound into helices which have opposite senses will have equal but opposite effects on the phase of the quantum state of circularly polarized photons. So why not take a single beam of photons, split it into two, direct each half into oppositely wound helical fibres and look for destructive interference? The difficulty is that of producing left and right-handed helices which are exact mirror images. Chiao and Akira Tomita (working then at AT&T Bell Laboratories) have instead measured the optical rotation that occurs when a linearly polarised beam of photons traverses an optical fibre wound in helices of different pitch (*Phys. Rev. Lett.* **57**, 937; 1986). Inevitably, Berry's prediction is confirmed.

Berry's argument goes to the root of a string of deep questions in quantum mechanics. There are obvious connections with the way in which the magnetic flux enclosed by a superconducting loop, a Josephson junction for example, is quantized. There are connections with Dirac's famous magnetic monopoles. A feature of Berry's construction is that the change of phase appears to be determined only by geometrical or, strictly, topological considerations. The phase shift generated by a single turn of a uniform helix is determined only by the path traced out on the surface of a sphere by the changing propagation vector. For a helix, this path is a circle, but it emerges that any other closed path (meaning any other fibre path) would have the same effect on the phase so long as the wave-vector traces out a closed loop enclosing a patch of surface on the same hypothetical sphere which subtends the same solid angle at the origin.

The other side of the coin is that both authors remark that their experimental results are those that would have been obtained classically, by using Maxwell's equations to calculate the intrinsic optical activity of a helically wound fibre. The novelty, not yet tested, is that Berry's result should obtain even with single photons. If that prediction prove correct it points to a novel correspondence between classical and quantum mechanics.

John Maddox

Environment research

Is lead pollution of the atmosphere a global problem?

from David A. Peel

LEAD pollution has been studied for decades, yet still there is controversy over whether large-scale contamination has occurred. Purely natural processes, such as volcanism and the weathering of rocks, release lead into the atmosphere. Some still believe that these sources dominate concentrations in the global atmosphere

and increases in mineral dusts and sea salts in the atmosphere accompanied the coldest part of the ice age. At the same time concentrations of lead in the Antarctic atmosphere were greater than at any time during the Holocene, including the present. It is therefore an ideal period to test our understanding of processes that contribute to natural levels of lead in the environment.

The results of this new, extremely careful piece of work show clearly that, within reasonable limits, the observed levels of lead in the prehistorical period can be accounted for simply on the basis of their average abundance in the Earth's crust. Although contributions from volcanic activity may have been similar to crustal contributions during the Holocene, they were evidently insignificant during the height of the Wisconsin. All this must undermine seriously the arguments of those who suggest³ that levels of lead from natural sources are enhanced by a range of exotic natural enrichment processes. Such processes have been invoked to account for orders of magnitude discrepancies between high measured levels of lead in the environment and predicted levels based on estimated emission rates from the various sources. The observation that several workers have been unable to detect increasing levels of lead during recent decades was also attributed to the existence of these large enrichments of natural sources. It is now apparent that these findings are much more likely to represent flaws in contamination control.

The new data are in accord with values previously published⁴ by the same laboratory for a few samples of both Greenland and Antarctic ice cores between 1,500 and 5,500 years old. The new mean concentrations (0.7×10^{-12} g Pb g⁻¹ ice) for Holocene ice from Antarctica can be compared with the most reliable data available for modern snow^{5,6}. This comparison suggests that there was a modest increase of up to sixfold in lead concentrations in Antarctic snow between prehistoric times and the present (Fig. 2), but there are insufficient data free from contamination to show a clear increasing trend through the industrial period.

The evidence from Greenland is little better and the most reliable time series, published in 1969⁷, is still cited widely. These data were a composite from three locations and systematic differences between the sites may have influenced the time series. Methods of sample collection

and contamination control have improved greatly since then. However, several authors independently have measured concentrations of around 200 pg g⁻¹ in present-day snow in Greenland, Alaska and the eastern Arctic Ocean. The best-documented values⁴ for ancient ice, obtained from three samples of a Greenland ice core (1,500–5,500 years old) are around 1.4 pg g⁻¹, a value essentially supported by the new data from Antarctica. There seems little doubt that lead concentrations have increased around 200-fold in Greenland snow since prehistoric times. The observed contrast in the response of the Greenland and Antarctic atmospheres to rising environmental levels of lead accords with the fact that lead emissions

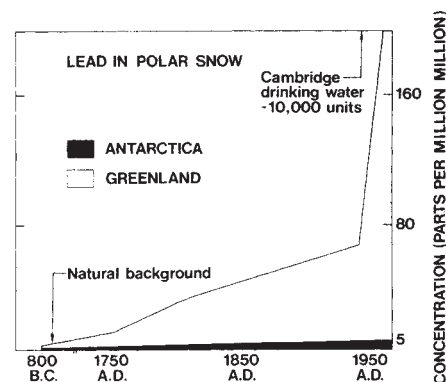


Fig. 2 Contrasting trends of Pb concentrations in Antarctic (first-order interpolation of available data) and Greenland⁷ snow.

occur predominantly in the Northern Hemisphere. There is effectively little transfer between the hemispheres, because the atmospheric residence time of lead aerosol in the troposphere is short in comparison with the time required for inter-hemispheric mixing.

Overall a generally blurred picture is emerging of increasing lead pollution in both the north and south polar regions, with a much more pronounced change in the Northern Hemisphere. There is now an urgent need for new and detailed time series to cover the past few hundred years in both Greenland and Antarctica at single sites remote from any form of human activity. The new data show that if meaningful time series are to be achieved, then the scientists involved must be able to demonstrate at the outset that they can make reliable measurements at the level of one picogram per gram. □

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7. Murozumi, M. et al. *Geochim. cosmochim. Acta*, **33**, 1247 (1969).

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Fig. 1 Sampling the Antarctic ice sheet for global pollutants (photograph by M. Vallance).

but there is a growing consensus¹ that lead derived from human emissions is dominant even in the remotest parts of the planet. The report by Boutron and Patterson on page 222 of this issue² contains the best data yet obtained for natural levels of lead in Antarctic ice through the peak of the last ice age (Wisconsin) and into the Holocene (see Fig. 1).

Fortunately, records of the changing composition of the Earth's atmosphere over thousands of years have been perfectly preserved within the ice layers of the polar ice sheets. Analysis of ancient ice can provide a vital reference for the natural state of the Earth's atmosphere and allow modern trends to be judged in proper perspective. Unfortunately, analyses are generally carried out in laboratories close to sources of lead pollution and, as a result, most reported measurements have been affected by contamination.

The data of Boutron and Patterson are from a period of dramatic change in the Earth's atmosphere, when massive in-

Perception

Vision in humans and computers

from Oliver Braddick

HUMANS extract information about objects and events from optical images with effortless efficiency. It is surprisingly difficult to specify exact algorithms that can do the same, but this is the task that the field of 'computational vision' sets itself. A recent symposium* reviewed advances in this area and their impact on our understanding of human vision.

The interaction between computational and experimental studies of vision is now taking place over a wide range of topics, albeit in rather different ways. In colour and motion perception, detailed modelling of initial detection processes has been well established for some time and is closely integrated with quantitative experimental work. For motion detection, G. Sperling (New York University) discussed the elaborated Reichardt model, which multiplies filtered versions of the signal from adjacent regions, whereas E. Adelson (RCA Laboratories) argued for a model that combined signals from receptive fields in quadrature phase to derive the 'spatio-temporal energy' present in a particular direction of motion. Both agreed that these models are, at least potentially, formally equivalent in their input-output relations, but that one or other would turn out more appropriate as a description of the biological system.

It was striking that, in motion as in other topics, the neurophysiological work now attracting most computational interest is not that on the primary visual cortex (V1), but on the multiplicity of higher cortical visual areas. The combination of motion signals from different parts of a contour is necessary to provide unambiguous directional information (E. Hildreth, MIT), and this may be a function of cortical area MT, known to be specialized for motion processing (J.A. Movshon, New York University). Cells in this same areas respond to differences in motion between the receptive field and the surrounding region (J. Allman, California Institute of Technology), which may be important for segmentation of the visual scene.

In colour as well as motion, broader computational issues arise from how initial detector measurements are combined to yield information more closely related to properties of objects in the outside world. An important goal of such combination is to disentangle information about surface properties from the effects of the illuminating light (colour constancy). As in many computational analyses, a key element is to discover the

physical constraints which the visual system can exploit to limit an otherwise intractable problem. Although both illuminants and surface reflectances can in principle have an infinite variety of spectral functions, in the real world 99 per cent of the variance in illuminants can be described by three variables, and a similar reduction is possible for the description of

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

a-c, Fractal brownian surfaces can be constructed by recursively adding smaller and smaller 'steps' to a smooth surface. The fractal dimension of a surface depends on the ratio between the altitude of the larger 'bumps' to that of the small bumps. *d*, Six different ratios, giving fractal dimensions between 2.0 and 2.5. Increasing fractal dimension corresponds very well to the perceptual notion of 'roughness'. (Courtesy of Alex Pentland.)

surfaces (B. Wandell, Stanford University). The problem of colour constancy still exists, for these six variables have to be computed from the three signals of trichromatic colour vision, and the process requires integration of information from surfaces at different locations. The neural basis for this process may be in cortical area V4 (S. Zeki, University College, London).

Area V4 is probably not exclusively concerned with colour processing. Neural responses in this area are modulated by the monkey's selective attention (R. Desimone, NIMH, Bethesda; J. Maunsell, University of Rochester). Cells in V4 have receptive fields large enough to contain several stimuli, but Desimone finds that attention to one of these narrows the field to that region only. Interestingly, this does not act to suppress the response of a cell entirely when its field contains only the single unattended stimulus; the effect seems to be intrinsically competitive. Work on selective attention might seem to be a long way from problems of purely visual computation, but S. Ullman (MIT) pointed out the important connection. Certain apparently simple visual properties (for instance, whether two marks lie on the same

continuous contour) cannot be readily computed by uniform parallel operations over the visual field but are handled much more naturally by serial 'visual routines' built up out of a few basic operations. One of the most important of these operations is 'indexing', or spotlighting a particular location within the visual field as the site of the current operation. Ullman's group is now gathering response-time data, which allow experimental tests of the sequences of basic operations used by human perceivers making simple spatial judgments.

The example of selective attention shows that computational models are certainly not restricted to the lower levels of visual processing, but can now be applied to problems such as texture, shape and space. Many visual textures can be modelled by brownian fractals (see figure), in which an exponent (the fractal dimension) relates the amplitude of features to their scale (A. Pentland, SRI International, Menlo Park). Pentland suggested that the visual system extracts this exponent by detecting linear trends in the spatial frequency spectrum, and that regions which differ in this exponent can be perceptually segmented. These interesting ideas have not yet been tested in experimental studies. The same appears to be true of work on how object shapes are subdivided into parts (D. Hoffman, University of California, Irvine). Hoffman's computational model, which locates part boundaries at negative extrema along lines of greatest surface curvature, seems to fit in many cases with intuition, but we lack psychophysical methods for systematically locating the perceptual boundaries of object parts. On the other hand, the novel work of R. Andersen (Salk Institute), on how neurones in a parietal visual area jointly represent retinotopic location and direction of gaze, highlights an issue of longstanding psychophysical and neurophysiological interest that has received little computational analysis.

The subjects of the symposium were diverse, but several themes recurred. Images contain information at various scales, and can often be dealt with efficiently by a 'pyramid' of scale-invariant processes whose capacity increases proportionately with the greater detail of the finer scales of analysis. Segmentation is an essential process, but one which must preserve the hierarchical relationship between a whole object and its component parts. Computational thinking is now intimately embedded in the study of the early processes of vision; in pursuing these principles and their implications it is making increasing contact with our rapidly expanding knowledge of the neural basis of higher visual processes. □

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*Computational Models in Human Vision Center for Visual Science, University of Rochester, 19-21 June 1986.

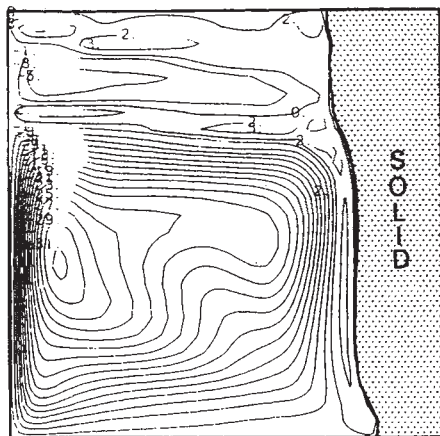
Crystal growth

From multi-branched snowflakes to precious minerals

from Herbert E. Huppert

MULTI-BRANCHED snowflakes, gallium arsenide crystals for use in integrated circuits and the olivine crystals that permeate the Earth's mantle are all formed by solidification. Can the understanding gained from carefully controlled industrial crystallization processes lead to clearer insight into naturally occurring crystallization? Or can industrial solidification be made more efficient by mimicking natural phenomena? These were among the questions considered at a conference* of some 60 crystal growers, geologists, mathematicians and metallurgists.

In many industrial processes, solidification of a one-component product from



Stream function

Fig. 1 The numerically calculated stream function and solid region produced when an initially uniform, 10 wt per cent aqueous Na_2CO_3 solution is cooled for 20 min at the right wall and warmed at the left (M.E. Thompson). One large cell has developed with most rapid flow at the two vertical edges, and two much weaker cells are evolving near the top of the container.

a multicomponent melt is induced by moving a heat sink at constant velocity along the outside of the receptacle containing the melt. If the velocity is too large, the planar solid/melt interface becomes unstable and leads to a disastrous, irregular form of solidification. In a quiescent melt, the velocity which determines instability to infinitesimal disturbances was evaluated by Mullins and Sekerka in 1964. A recent numerical study by R.A. Brown (Massachusetts Institute of Technology), which allows for large departures from a planar interface, shows that at suitably rapid solidification velocities a cell generated by the initial linear instability can bifurcate into two cells of half the wave-

length, each of which can bifurcate again and again, until a field of stable dendrites of sufficiently small wavelength evolves. It appears that parameters for the cooling of large bodies of magma within the Earth's crust are such that an interface dominated by denrities almost always results.

Evaluations of the paradigm shape of a single, smooth dendrite and its inherent instability are evoking considerable controversy at the moment. A steady-state model by G.P. Ivantsov, which neglected surface tension, does not predict a unique solution, but merely a family of solutions. J.S. Langer determined that dendrites could be unstable to side-branching and suggested that a dendrite propagates at the velocity of marginal stability. This seems to be in good agreement with experiments. But why? This question has vexed many physicists who are developing quite complicated but rather *ad hoc* models that attempt a resolution by appealing to special effects such as the anisotropy of surface tension. A different approach was described by J.J. Xu (Rensselaer Polytechnic Institute). He considered the axisymmetric dendrite as a slender body (an idea first pioneered in the study of flow past airplane fuselages which has now been applied to the study of the motion of ships and the growth of roots of plants). Xu determined the form of solution close to and far from the dendrite and related these two solutions using the technique of matched asymptotic expansions to derive a single integro-differential equation for the growth velocity. For zero surface tension, the original one-parameter family obtained by Ivantsov is retrieved. With the inclusion of surface tension, the equation has a solution only for growth velocities less than a critical value; beyond this critical value no solution exists. The relationship between this critical velocity and the velocity of marginal stability has yet to be determined.

As dendrites grow, they develop secondary, tertiary and higher-order branching. With time, the solid protrusions became more rounded due to the Gibbs-Thomson effect, which states that the local temperature at a curved solid/liquid interface is depressed from the freezing point because of the capillary effects of the curved interface by an amount proportional to γ/Γ_1 , where γ is the surface tension and Γ_1 the total local curvature. Fluxes of heat then occur resulting from the temperature gradients between interfacial regions of differing curvature and these fluxes induce

preferential melting and change of shape and size. Generalizing this idea in a statistical sense to a complicated two-phase mixture suggests that the mean undercooling, $\langle \Delta T \rangle$, is proportional to $\gamma/\bar{\Gamma}$, where $\bar{\Gamma}$ is the average curvature. Theoretical analysis indicates that the size of particles evolves with time t resulting from the different fluxes between adjacent interfaces with $\langle \Delta T \rangle \propto \bar{\Gamma} \propto t^{-1/3}$ for large time. Experiments by M.E. Glicksman (Rensselaer Polytechnic) in several partially molten systems including succinonitrile, ethylene carbonate and ice/water, which solidify with different morphologies, verify this relationship and for the first time allow the surface tension coefficient to be determined simply and directly. The evolution to sphericity of snowflakes in a snow field in the timescale

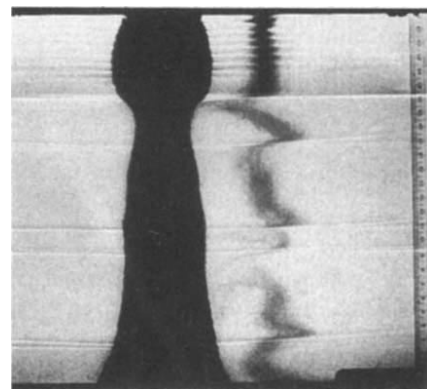


Fig. 2 The result of cooling for 22 h an initially homogeneous, almost saturated, aqueous Na_2CO_3 solution at a thin central rod (J.S. Turner). Five large cells have developed at the base with nine thinner cell above, as seen by the double-diffusive interfaces and the KMnO_4 dye trace initiated a few seconds before the photograph was taken.

of weeks is an example of this process.

Solidification of a multicomponent melt is often dominated by fluid effects, in particular convective phenomena, because the solid rejects part of the melt, which is of a different density. Considerable interest has been shown recently in various fluid processes by geologists who wish to apply the concepts to understand the solidification of magma and to predict the formation of precious minerals such as chromium, nickel and platinum. I summarized comparisons between recent laboratory experiments and theoretical calculations predicting the fluid flows and rate of production of the solid. One particular case — cooling and solidifying a multicomponent melt from the side — was considered further by A.M. Leitch (Toronto) and M. Thompson (MIT). Leitch has conducted experiments with different aqueous solutions (of, for example, Na_2CO_3 , FeSO_4 , KNO_3 , NH_4Cl). In all cases the buoyancy difference of the released liquid at the solid/liquid interface induces a vertical convective flow. Because of the finite volume of the experi-

* *The Structure and Dynamics of Partially Solidified Systems* Lake Tahoe, California, 12–16 May 1986.

mental container the released liquid pools at the top or the bottom of the container and causes a strong vertical density stratification to be set up. In the experiments the rate of solidification and the vertical mass transfer rate does not vary much, although the crystal habit and roughness of the wall changes considerably. Thompson has numerically calculated the flow fields corresponding to this two-dimensional situation (Fig. 1), which are also in qualitative agreement with laboratory observations of an axisymmetric experiment conducted by J.S. Turner (Australian National University) (Fig. 2). M.G. Worster (Cambridge) analysed the cooling of a subeutectic binary alloy from below, and extended the earlier model that he and I derived based solely on global conservation relationships to predict local variations of the solid fraction; both models yield predictions quite close to those observed in the laboratory.

A.R. McBirney (Oregon) was one of

several geologists who described the consequences of crystallization of slowly cooling bodies of molten rock and the interpretation of the final products of this process long after the solidification is complete. He described how differentiation caused by a process akin to metallurgical zone refining could be responsible for strong fractionation in large intrusions, and suggested that residual products of crystallization sweep through the crystal mush, fluxing earlier crystals and collecting the excluded components in a zone of water-rich melt. I.H. Campbell (Australian National University) and J. Peterson (Aarhus) proposed other processes to explain various puzzling features of slowly crystallizing magmas, such as thick sequences of very uniform rocks that have crystallized on the walls and floors of large intrusions. □

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Lectin biochemistry

New way of protein maturation

from Nathan Sharon and Halina Lis

WHEN last year it was claimed that the mature form of the jack bean lectin concanavalin A (Con A) is made by the breakage and rejoining of its precursor peptide¹, some scepticism was voiced in a *News and Views* article². This was not surprising as a novel form of protein maturation had been invoked. Doubts should be quelled, however, by a more recent paper³ that also suggests transpeptidation is the mechanism involved.

Con A, which consists of four identical subunits of relative molecular mass (M_r) 27,500, is a member of the family of legume lectins (including, for example, lentil lectin, soybean agglutinin and fava bean lectin), all of which show extensive sequence similarities when properly aligned. But a puzzling feature of Con A (and of another lectin recently isolated from *Dioclea grandiflora*) has been that maximum similarity with the other legume lectins only occurs when its amino terminus is positioned near the middle of the polypeptide chains of the other lectins (Fig. 1).

Although it has been suggested that reorganization within a common ancestral gene of the legume lectins is responsible for this unusual feature of the Con A sequence^{4,5}, the report published last year by Carrington *et al.*¹ shows, surprisingly, that the amino-acid sequence deduced

from complementary DNA derived from the messenger RNA encoding Con A compares directly with the other legume lectins. The DNA contained a coding region corresponding to 29 amino-acid residues of a signal sequence, followed by a region corresponding to amino acids 119–237 of Con A, a region encoding 15 amino acids not found in the mature lectin, and finally a region corresponding to amino acids 1–118 of the lectin, followed by a 9-residue carboxy-terminal extension. To explain the sequence found in

the mature lectin, Carrington *et al.* suggested that during formation there must be a transposition and ligation (between residues 118 and 119) of two peptides produced from the precursor polypeptide.

Two recent reports by D.J. Bowles and her colleagues^{3,6} provide a new look at the

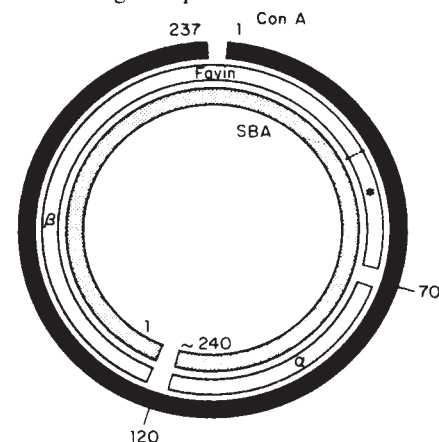


Fig. 1 Alignment of fava bean lectin (favin), soybean agglutinin (SBA) and Con A, showing the circular permutation that gives maximum homology among the sequences of these lectins. (From ref. 9.)

mechanism of Con A biosynthesis. In studies of the formation of the lectin during embryogenesis by ¹²⁵I-substituted Con A overlays and Western blotting, they obtained evidence that a putative precursor of the lectin (M_r 33,500) was glycosylated⁶. The formation of an N-glycosylated Con A precursor was also observed by Herman *et al.*⁷. Mature Con A is not a glycoprotein and does not contain any potential glycosylation sites. However, as pointed out by Herman *et al.*, there is a potential glycosylation site (Asn-Ser-Thr) in the 15-amino-acid insert mentioned above. These authors suggested the removal of a glycopeptide as the sole processing step between the glycosylated precursor and the final lectin form.

The studies by Bowles *et al.* on the post-translational processing of Con A, using metabolic labelling of immature jack beans and pulse-chase experiments, reveal a more complex series of events⁷. They show that the glycosylated M_r 33,500 precursor is first deglycosylated and then converted into two peptides of M_r 18,800 and 14,200. Surprisingly, after continued chase, there is a gradual disappearance of the low M_r peptides and the appearance of a new entity of M_r 30,400, suggesting ligation between the two fragments of low M_r . Amino-terminal sequence analyses revealed that the alignment of residues 1–118 and 119–237 was reversed in the mature lectin from that of the precursor first label-

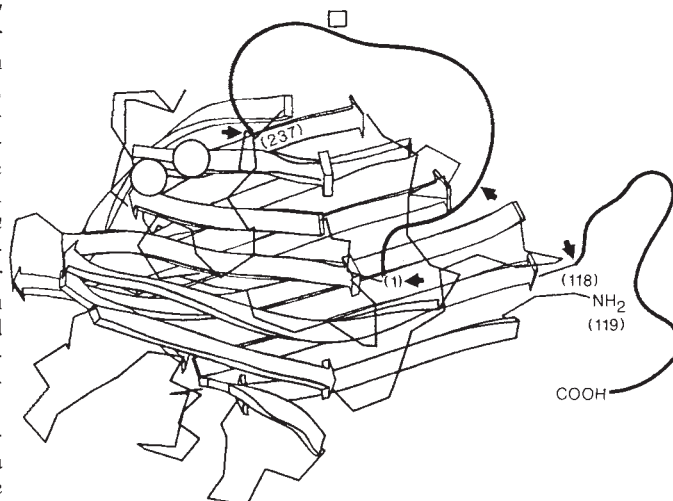


Fig. 2 Hypothetical three-dimensional structure for the M_r 33,500 precursor of Con A fitted with a computer model of the structure of the mature lectin. Numbers correspond to residue positions in mature Con A. Arrows indicate the approximate positions of proteolytic processing. The proposed location of the oligosaccharide side chain is also marked (□). (From ref. 3.)

led. These findings demonstrate a novel means of assembling a mature protein, involving the rearrangement of a primary sequence by the post-translational formation of a peptide bond, as proposed by Carrington *et al.*¹. The function of the ligation is not clear as the carbohydrate-binding activity of Con A can already be detected after the precursor molecule is cleaved into the two fragments³.

As the chase data imply that the formation of the peptide bond might occur simultaneously with the proteolytic removal of the carboxy-terminal extension at residue 118, Bowles *et al.* propose that annealing of the fragments to produce the mature lectin involves a transpeptidation rather than proteolysis and subsequent ligation. A well-known example of peptide bond formation by transpeptidation (a reaction that does not require an external source of energy) is the last step in the synthesis of bacterial cell wall peptidoglycan⁶. However, this type of reaction has not been encountered in higher organisms.

Bowles *et al.* have further demonstrated by computer modelling that it is possible

to fit the M_r 33,500 precursor with the three-dimensional structure of mature Con A. The 15-residue insert forms an extended surface loop and the 9-residue carboxy-terminal extension sticks out from the surface (Fig. 2). In this model, residues 118 and 119 of the precursor are already positioned reasonably near each other on one surface of the protein. The entire series of processing events could thus occur without significant refolding of the initial translation product. □

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Classical mechanics

Tops that like to spin one way

from Robert Walgate

COULD a rigid, solid object like an asymmetrical marble be shaped to spin in one direction on a hard, smooth table, but not the other? The answer is yes, but it has taken nearly a century since the phenomenon was first reported (Walker, G.T. *Q. Jl pure appl. Math.* **28**, 175; 1896) to discover the precise reasons why (Bondi, H. *Proc. R. Soc. A* **405**, 275; 1986).

Hermann Bondi's lengthy classical analysis of what is effectively the rolling motion, with spin, of an asymmetrical top was prompted by the gift of a boat-shaped toy (see figure). When spun by hand on the table-top in one direction, the boat spins freely; but in the other, it consistently shudders to a stop and then reverses its spin. Bondi's Cambridge colleague Brian Pippard has long demonstrated to students, though not fully analysed, an even more peculiar effect, when a spinning, highly polished, stainless-steel object of suitable shape reverses its direc-

tion of motion if spun either way on a stainless-steel plate. Here the handedness appears differently: spun in one direction the body goes into sluggish, sideways oscillations, and then reluctantly reverses; spun in the other direction it becomes grossly unstable and reverses more rapidly. Bondi's new analysis also explains this motion.

The complete argument is complex, involving the movement of the roots of a characteristic equation during the motion of the object, but the key insights are elegantly simple. They explain two apparent paradoxes: the non-conservation of angular momentum in the object; and the emergence of chirality, or handedness, although the laws of classical mechanics are evidently not chiral.

Angular momentum is a simple issue. The angular momentum of a body about its centre of mass can only change as a result of couples exerted on the body by external forces. With a rolling body, the only such forces are gravity (which, acting at the centre of mass, has no couple about it) and frictional forces at the point of contact. We are familiar with cases (for example a rolling sphere) where the point of contact is directly under the centre of mass so there is no couple about the vertical, or other cases where this couple fluctuates rapidly about zero mean. In the case Bondi describes, the couple has con-

sistently the same sign, and thus systematically alters the angular momentum of the body.

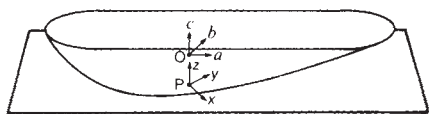
The origin of the chirality of the system is more difficult, however. Both Bondi's and Pippard's spinners are twisted, like a screw, although the twist in Bondi's spinner is almost too slight to be noticeable. But how does the twist affect the dynamics? The key parameter of twist, Bondi shows, is one between the three perpendicular principal axes of inertia of the spinner, and the axes of its presumed ellipsoidal surface of contact with the table. The axes of inertia affect how the rotational kinetic energy of the body about its centre of gravity alters with the angular velocity of the body; the contact axes affect how its gravitational potential energy (governed by the height of the centre of gravity) varies with angular position or roll. The contact axes also affect the velocity of the centre of gravity, and hence the linear kinetic energy of the body, as a function of angular velocity about the contact point. The relationships of the axes thus determine a complicated feedback relationship between the various forms of energy of the body, and thus affect its motion.

Moreover, it is theoretically possible to define a finite rotation, and an axis of rotation, which will rotate one set of perpendicular axes into another. Because the axes of inertia and the contact axes are physically different sets, a rotation from one to the other is physically different from a rotation in the opposite direction, so the object has a distinguishable chirality. Given that chirality between the axes, and in turn the intimate involvement of the axes in the motions of the object (as described above), it is not after all surprising that the objects should show chiral motions, as Bondi's detailed analysis proves.

In principle, even an asymmetrical pebble might show chiral dynamics, but frictional dissipation can soon damp out the motions before chirality is evident, Bondi says. The effects are increased to the degree to which the object is inertially 'oblong', the degree to which its surface of contact is geometrically elliptical, and according to the degree of skewness of the two systems with respect to one another. The effects are also sharper the more curved the contact surface.

Asked to draw moral from this modern revival of classical mechanics, Bondi quoted the opening words of his lecture on relativity: "This theory [relativity] has the reputation of being difficult; but in fact, Einstein's contribution was very simple. Unfortunately, relativity rests on the theories of Galileo and Newton. These theories are very difficult, but suffer from being thought easy. . .". □

Robert Walgate is European correspondent of Nature.



Bondi's one-way spinner at rest. O, centre of gravity; P, point of contact with the table; a, b, c, principal axes of inertia; x, y, z, axes of the quadric most closely approximating the surface of the spinner at P.

Neurobiology

Dopamine receptor disputes

from J. M. Palacios

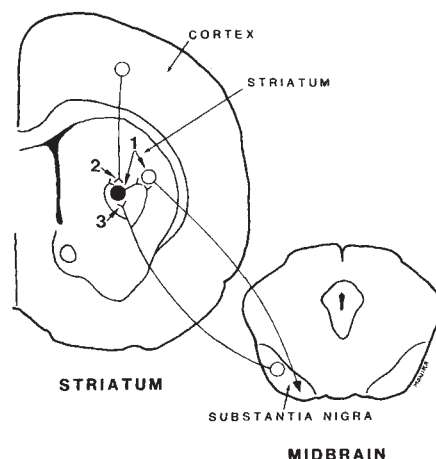
'ESTABLISHED' ideas have a difficult time in neurobiology. Once again one of them, the question of the localization of dopamine receptors in the terminals of cortical neurones projecting to the striatum, is contested in a paper by J.M. Trugman *et al.* in this issue (*Nature* 323, 267; 1986). Brain receptors for the neurotransmitter dopamine, particularly those of the D₂ type, mediate the action of many anti-psychotic drugs and anti-parkinsonian agents. To know the exact cellular localization of dopamine receptors will help to elucidate the mechanisms whereby anti-psychotic drugs exert their effects, and how these effects are channelled in the brain.

Recent progress in elucidating the site of dopamine receptors include their direct visualization in living human subjects using positron-emission tomography (Wagner, H.N. *et al.* *Science* 221, 1264; 1983). Although some brain areas, mainly basal ganglia nuclei such as the caudatus and putamen, are particularly enriched in D₂ receptors, we cannot yet determine in which cells, or where in the cells, they are located.

In the absence of probes to visualize directly dopamine receptors at the cellular level, biochemical assays of receptors, for example radioligand binding assays, are combined with the placement of neurotoxin or mechanical brain lesions (Schwarcz, R. *et al.* *Nature* 271, 766; 1978). Electrophysiologists also painstakingly try to determine the pre- and post-synaptic sites of action of dopamine. Their results are in general compatible with biochemical results (see, for example, Mercuri, N. *et al.* *Brain Res.* 358, 110; 1985). These kinds of experiments led to the generally accepted idea that dopamine D₂ receptors in the striatum are localized in: (1) the intrinsic neurones such as the interneurons that use acetylcholine as a messenger or the medium spiny neurones; (2) the axon terminals of a projection from the cortex that uses glutamate as transmitter (the cortico-striatal pathway); and (3) the terminals of the dopaminergic neurones themselves (presynaptic autoreceptors) whose cell bodies are located in the substantia nigra, a nucleus of the mid-brain (see figure). This scheme is, however, based exclusively on circumstantial evidence and is subject to the many limitations of the techniques used.

This apparently well-established scheme is now contested by the paper of Trugman *et al.* in this issue. Using experimental procedures similar to those described above (a combination of specific

lesions with binding assays) they conclude that most D₂ receptors in the rat striatum are in the intrinsic striatal neurones and not equally divided between these cells and the terminals of the cortico-striatal pathway. The difference between these new results and previous ones arises from the use of quantitative receptor autoradiography, a technique (Young, W.S. & Kuhar, M.J. *Brain Res.* 179, 255; 1979) allowing visualization of receptors at the level of resolution achievable with the



Proposed localizations of dopamine D₂ receptors in the rat striatum. (1) Intrinsic neurones; (2) nerve terminals of the cortico-striatal pathway; and (3) nerve terminals of the nigro-striatal pathway.

light microscope. Trugman *et al.* created lesions in the cortico-striatal pathway by suction of a small cortical area known to contain the cells of origin of the terminals in the striatum. These lesions produce degeneration in the striatum but do not affect the density of D₂ receptors. In contrast, when the toxin kainic acid is used to destroy the intrinsic neuronal cell bodies, but not afferent terminals, there is a large decrease of D₂ receptor binding in the lesioned tissue.

How can the conflict with previous results be explained? Use of microscope methods allows the precise delimitation of the extent of the lesion and the calculation of the decrease of receptors in the area where neurones died and not in the unaffected remainder of the brain nucleus. This is difficult to control in biochemical and electrophysiological studies, and has a general bearing on the use of brain lesions in biochemical studies without appropriate histological control of the extent of the lesion.

Although provocative, the results in the paper of Trugman *et al.* do not solve the problem of the localization of dopamine receptors in the brain because of the lack of ultrastructural resolution. Only the development of probes such as antibodies directed against purified receptors or genetic probes will allow more precise identification of the cells that make dopamine receptors. Complete elucidation of the role of dopamine receptors in normal and psychotic brain function awaits further technical advances. □

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Parasitology

Improving prospects for a schistosomiasis vaccine

from S.R. Smithers

AMID the expectations for the imminent development of a malaria vaccine, several laboratories have been working steadily towards a vaccine that will protect against another important human parasitic infection, schistosomiasis. Developments were reviewed at a recent meeting* and the consensus was that vaccination against schistosomiasis in man is an attainable goal.

Man becomes infected through contact with fresh water containing infective cercariae shed from intermediate snail hosts. These penetrate exposed skin and rapidly transform to larval schistosomula that are physiologically adapted to live within the body. The schistosomula migrate

through blood vessels to the lungs, then to the liver, where they mature into adult worms, and finally to the blood vessels of the intestine (*Schistosoma mansoni*) or bladder (*S. haematobium*), where each male-female pair can produce several hundred eggs a day.

It is widely thought that the control of schistosomiasis by the drug praziquantel is so successful that no further measures are required. This view, however, has been sharply challenged. Reinfection can occur rapidly, particularly among children, who contribute most to parasite transmission. Treatment must therefore be repeated frequently, which increases costs and makes this form of control less attractive. Also, schistosomes resistant to an earlier drug, oxfamiquine, have been recorded

*Prospects for Immunological Intervention in Human Schistosomiasis Geneva, Switzerland 26-28 May 1986.

(Dias, L.C.S., Pedro, R.J. & Deberaldini, E.R. *Trans. R. Soc. trop. Med. Hyg.* 76, 652; 1982) and there appears to be no reason why praziquantel-resistant parasites should not develop under present conditions. Thus there is sound justification for the pursuit of a vaccine.

This pursuit is encouraged by the now clear evidence that man develops resistance to infection. Two separate studies measured the intensity of reinfection after drug treatment and revealed a marked association between increasing age of the patient and an enhanced resistance to reinfection that is independent of differences in the level of exposure to the parasite. In patients infected with *S. haematobium*, resistance correlates with high numbers of eosinophils and high titres of antibody to adult worm antigens. With *S. mansoni*, susceptibility to reinfection correlates positively with high levels of antibody recognizing schistosome egg antigens. This suggests that the initial immune response is somehow biased towards egg antigens and impedes the development of immunity, and may help to explain the slow development of resistance.

Work using *S. mansoni* on experimental animals suggests how this might occur. Infected animals develop immunoglobulin M antibodies to carbohydrate antigens found on both eggs and schistosomula (the target of immunity) that can block killing. Killing normally occurs in the skin shortly after cercarial penetration, by means of antibody-dependent cellular cytotoxicity. Later, during migration through the lungs or other capillary beds, schistosomula are no longer susceptible to surface damage, and attack relies on inflammatory responses that impede normal migration and development.

Although carbohydrate antigens that block effective immunity have been described, monoclonal antibodies have been used to define both carbohydrate and polypeptide antigens that are able to induce a protective immune response. The carbohydrate antigens not only cross-react with the egg stage of the parasite but also with other species of schistosome and are associated with schistosomula surface molecules of relative molecular mass (M_r) >200,000 (200K), 38K and 17K. The antibodies recognizing polypeptide determinants are species-specific and recognize surface molecules of M_r 32K, 28K, 22K and 20K shared by schistosomula and adult worms.

Mice and rats have been challenged with purified *S. mansoni* surface antigens of M_r 22K and 28K and an anti-idiotypic antibody raised against one of the carbohydrate-recognizing monoclonal antibodies. Levels of protective immunity attained in mice range between 20–40 per cent but are twice as high in rats. Immunization with crude fractions has also been

successful and has achieved levels of immunity up to 89 per cent in the rat and 70 per cent in the mouse. Similar work shows that non-surface proteins can also be protective and these include an antigen of M_r 97K which, from the sequence of the cloned gene, is identified as schistosome paramyosin.

The genes encoding the M_r 28K and 38K surface antigens and a gene encoding a 25K antigen present in the surface membrane of adult worms and lung-stage parasites have all been cloned in *Escherichia coli*. Two of these genes have also been inserted into vaccinia virus and their immunogenicity is under investigation. It appears that various molecules are potential vaccine candidates but it is likely that a 'cocktail' of antigens providing protection throughout the migratory phase of the life cycle will turn out to be the most effective candidate.

Geophysics

Causes of heating on the San Andreas fault

from Roger N. Anderson

WHAT is happening to the frictional heat that should be produced by the high stresses and movement of the San Andreas fault? Fascinating new results from the first deep measurements by Lachenbruch and his colleagues¹ in a 2-km oil company dry hole between Los Angeles and Barstow have at last shown a 40–50 per cent increase in vertical heat flow near the fault (see figure). This may be the extra heat that was expected from friction along a high-stress fault, but which was not found even after dozens of shallow measurements. But the heat flow is nonetheless puzzling, as it seems considerably lower in the surface sedimentary rocks than in the underlying granite.

There are certainly good grounds for expecting an increased heat flow. Frictional faulting theory and laboratory-derived coefficients of friction indicate high-strength crustal rocks. Large stresses of more than 1,000 atmospheres (1 kilobar) should be required to move rock along a transform fault such as the San Andreas. In support of these laboratory tests, Zoback² and colleagues had conducted numerous measurements to 1 km depth that suggest that indeed high stresses exist at shallow depths along the San Andreas fault. Such large stresses, if they can be extrapolated to depth, would produce enough frictional heat along the fault zone to produce a large and easily measurable heat flow anomaly, so it is reassuring that such has now been found, at least at depth.

The problem is how to explain the dif-

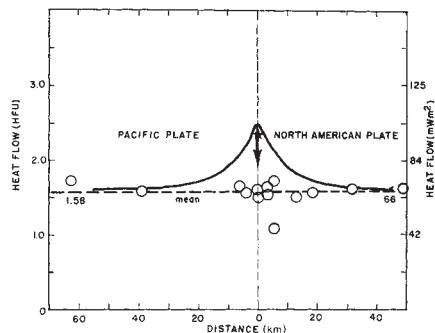
Clearly, progress in providing the molecular basis for a schistosome vaccine is now rapid and follows the lead the malaria field. Indeed, several problems of malaria are not found with schistosomiasis. For example, the schistosome does not multiply within the vertebrate host and therefore a completely effective vaccine may not be essential.

Unlike human malaria, schistosomes infecting man can be grown readily in experimental animals, facilitating the direct testing of a vaccine. Finally, the problem of antigenic diversity and variation, a feature of malaria infections, does not apply to schistosomes, where surface antigens appear to be highly conserved within a species. □

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ferential heat flows between the basement granite and the sediment above it. New observations show the profile of temperature against depth in the oil-company hole to be completely uniform and straight.

Heat flow is deduced from indirect measurements of thermal conductivity, which suggest that the sediment is considerably less thermally conductive than the basement. With the same temperature gradient in both structures, the heat flow must be less in the sediment than the basement, and either horizontal hydrothermal flow is carrying heat away near the boundary (which is only a few feet thick) or thermal refraction from steeply dipping



Heat flow versus distance away from the San Andreas fault. High-stress fault zone models predict high heat flow (solid line). The mean heat flow across the fault is flat (dashed line). Non-equilibrium, high heat flow at the fault, which is detected in a 2-km hole in basement granite, is indicated by the star. Corrections caused by complexities at the site may decrease the value in the direction of the arrow. Adapted from data in ref. 5.

contacts between the granite and sediment is deflecting the heat.

The argument thus puts a question mark over the calculations of thermal conductivity, but these are difficult to budge. First, geophysical logs³ indicated that the sediment — which is itself made of weathered granite — has 20 per cent porosity; and second, measurements of electrical conductivity show that the basement rock is twice as electrically resistive as the sediment. Only fresh water, filling the porosity in the sediment, is electrically conducting enough to explain the difference. The assumption of 20 per cent fresh water then brings the calculated thermal conductivity of the sediment well below that of the basement.

It thus appears that the heat flow around the oil well is complex, possibly suffering from local disturbances. The region is certainly geologically complex. The hole is in a mountain pass, and Ray Weldon of the US Geological Survey has shown from careful, skilful geological mapping⁴ in the area that about 1 km of sediment has been eroded from the well site in the past million years. Calculations by Lachenbruch show that this amount of rapid erosion could cause some, but probably not all, of the anomalously high heat flow in the granite.

The question now is what is happening to heat flow, and the supposed high stress in the fault, even deeper than the 2 km presently drilled. Luckily, observations at those depths may soon be to hand. Some while ago, Mark Zoback proposed to drill a new 5 km hole at the site of the oil-company hole at a time when the 'heat flow anomaly' was the total absence of frictional heating.

Now, this deeper hole, to begin in 1987, seems even more imperative. Zoback will lead a team of 27 scientists from 7 universities and US Geological Survey in making geophysical and geochemical measurements in the hole, which is to be sponsored by the US National Science Foundation programme for the deep observation and sampling of the Earth's continental crust (DOSECC).

The DOSECC programme will also receive core which will be made available for general study by scientists, in much the same fashion as the Ocean Drilling Program supplies its deep-sea cores. Only this deep work below the apparent 'disequilibrium zone' at the drill site seems likely to provide the deep measures of stress and heat flow near the San Andreas fault that are now required. □

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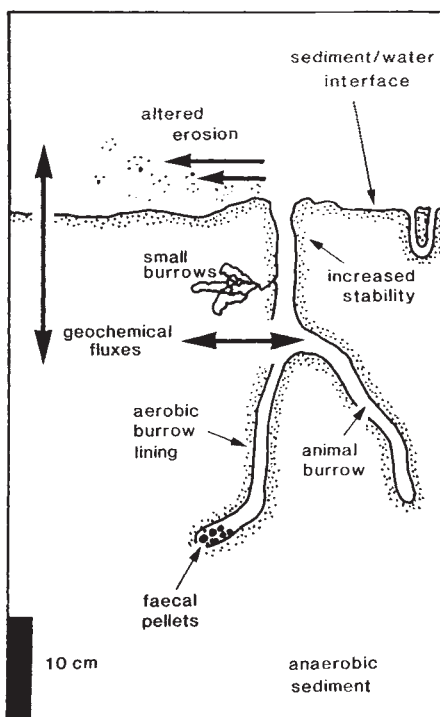
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Marine biology

Biological activity and seabed sediment structure

from P.S. Meadows

THE top metre or so of all sediments in the sea contains a rich diversity of animals and microorganisms. Animal burrows can increase the surface area of the sediment-water interface by a thousand-fold or more, and microbial activity can cause sediments to become totally anoxic. Re-



cent reports suggest these activities are an integral part of the sediment fabric, inhibiting sediment erosion and controlling the complex patterns of early sediment diagenesis and the first stages of oil formation^{1,2}. Aller's³ elegant experiments show that geochemical fluxes of inorganic ions can be dramatically altered by the presence of burrow structures, and highly significant chemical and microbiological changes have been described in shallow and deep sea sediments *in situ*^{4,5}. The report by King on page 257 of this issue⁶ of the inhibition of microbial activity in sediments by 2,4-dibromophenol (DBP) from the hemichordate *Saccoglossus kowalewskii* shows further the significance of biological activity on sediment structure.

S. kowalewskii secretes high concentrations of DBP in its mucus which then become incorporated into the lining of the animal's burrow. The burrow lining is aerobic because *S. kowalewskii* ventilates its burrow, whereas sediment nearby is anaerobic. Now King shows that DBP

markedly inhibits aerobic microbial activity that would otherwise occur in the burrow lining, but has little effect on nearby anaerobic microbial activity. He therefore deduces that DBP is important in increasing the longevity of the mucus lining of the burrow wall, hence maintaining the integrity of burrows in the sediment. He also argues that the inhibition of aerobic microbial activity will lead to the deposition of iron oxyhydroxides — and these are indeed found in quantity in the burrow linings of this species.

These findings support further the key role of biological activity in the chemistry and physics of the top metre or so of marine sediments^{7,8}. The general picture now emerging is of an interacting system between macrobenthic and meiobenthic animals burrowing in sediments, changes in microbial biomass and activity⁹, and altered chemical and physical sediment properties (see figure).

Although this picture is emerging, we still know surprisingly little about marine sediments. *S. kowalewskii* lives in the relatively accessible intertidal regions, and the sediments that cover most of the seabed are documented only in the broadest outline. Little is known about the influence of bioturbation and microbial activity on sediment erosion and transport, and even less about their effects on geochemical fluxes in and across the sediment⁷. Adequate models need to be developed from Berner's¹⁰ classic mathematical approach.

The potential significance of this work cannot be overemphasized. It is central to our understanding of such diverse aspects of sediments as the chemistry of early diagenesis and oil formation, and the control of sediment stability in harbours and estuaries and around man-made structures on the seabed. □

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Dragon's exhalations give clue to Chernobyl

SIR—Reading the letter from Liverpool University concerning the origin of $^{110}\text{Ag}_m$ in beef and lamb liver (G.D. Jones *et al.* *Nature* 322, 313; 1986) I was reminded that this isotope was well known as one of the more mobile fission product species in the ceramic fuels of the prototype high temperature gas-cooled reactor (Dragon) at Winfrith. It was produced, not from ^{110}Cd , but by epithermal neutron capture on ^{109}Ag . The yield was greater in ^{239}Pu fission than in ^{235}U fission but it was a prominent mobile species in both cases. It appears that $^{110}\text{Ag}_m$, whilst less volatile than ^{137}Cs in the elemental state, is capable of relatively rapid surface diffusion in graphite. Consequently, the gases released from the burning moderator graphite at Chernobyl would be expected to contain some $^{110}\text{Ag}_m$.

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Decontamination puts meat in a pickle

SIR—Meat from game, cattle, sheep and reindeer is still heavily contaminated with ^{134}Cs and ^{137}Cs from the Chernobyl nuclear accident. Scandinavia presents a particular problem, especially the reindeer in Lapland, but there is also contamination elsewhere in Europe. Since caesium, like sodium, is an alkali metal, we have attempted to substitute sodium for ^{137}Cs by pickling contaminated venison.

A leg of venison from a roebuck which was shot in the Schwaebische Alb mountains near Tübingen on 1 June 1986 contained 570 Bq (15.4 nCi) ^{137}Cs per kg, which corresponds to 178 pg (1.3 pmol) of radioactive caesium per kg meat. The leg was put into eight times its weight of a brine containing 100 g per litre (1.7 M) NaCl and 3 g per litre (29.7 mM) KNO_3 . After three weeks, the radioactivity of the meat had dropped to 70 Bq (1.9 nCi) per

kg (and it tasted very good). Similar results were obtained with livers, kidneys and hearts from two other roebucks.

Further investigation showed that potassium is a dispensable constituent of the brine. A typical example of extraction with NaCl alone is shown in Fig. 1. One kilogram of roebuck leg, containing 218 Bq ^{137}Cs , was cut into approximately fifty pieces to which were added 100 g crystalline NaCl and 500 ml 1.7 M NaCl solution. Each day thereafter, the meat was removed from the brine and washed with 100 ml water. The radioactivity of the meat and the brine was measured and the meat was repickled in a litre of 1.0 M NaCl solution. After five days the meat contained less than 5 per cent of the initial radioactivity.

We assume that meat from other animals could also be decontaminated by pickling with a large excess of sodium ions over caesium ions, of the order of magnitude of 10^{12} in this case.

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Body in the bog but no DNA

SIR—The isolation of partially degraded DNA from dried tissue of a museum specimen of the extinct quagga (*Equus quagga*) and its hybridization with DNA from the closely related zebra (*E. zebra*)¹ stimulated wide and sometimes wild speculation on the prospects of, genetically speaking, "raising the dead and buried". Commenting in News and Views on this important work, Jeffreys opened the challenge to museum curators to "be reasonably sympathetic to hordes of molecular biologists eager to dismantle their cherished exhibits" and alluded to the possibility of sequencing DNA from Egyptian mummies and from bog-bodies².

Since then, cloning of a small fragment of DNA from one of 23 Egyptian mummy samples examined has been described³. With the discovery in 1984 of the British bog body — Lindow Man⁴ — the opportunity arose to attempt to isolate DNA from peat-preserved human remains, although after about 2,500 years in an aqueous environment of pH 4.5–5.5, and with the expected rate of depurination, little if any undegraded DNA was anticipated to be present.

Samples of psoas muscle were removed for DNA analysis at a very early stage in the conservation of the body and stored in sterile containers at -60°C with maximum precautions to avoid contamination with modern microbial DNA. We first attempted but failed to isolate DNA following well-established methods, examining the

product by gel-electrophoresis, restriction enzyme digestion and hybridization to ^{32}P -labelled human ribosomal RNA. Then, in order to detect DNA degradation products such as oligonucleotides or apurinic acid, cell extracts were treated with alkaline phosphatase and then incubated with high specific activity $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ and T, polynucleotide kinase, but again without success. Similar results were obtained in experiments with peat samples taken from around this body during excavation, while fresh human tissue produced the expected DNA and its degradation products.

Thus, while a little DNA may be preserved during the dehydration process of Egyptian mummies, probably none survives the acid, aqueous, anaerobic and virtually sterile preservation conditions of bog-bodies, and hope of cloning the genes from Iron Age Man will probably not be fulfilled.

We thank the University of Liverpool research fund for support for this project, full details of which will be published elsewhere, and the British Museum, London for making tissue samples available.

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Out of Africa — through a genetic bottleneck

SIR—Giles and Ambrose¹ are correct to say that the phenetic analysis of β -globin gene data by which Wainscoat *et al.*^{2,3} and others⁴ have grouped Africans apart from other human populations should not have been interpreted as a phylogeny, because of ambiguous character polarities. Jones and Rouhani⁵ agree that the assumed polarities in their model of a genetic bottleneck are arbitrary, but offer no means of resolving the ambiguity. But by examining the β -globin gene clusters of the great apes it may still be possible to ascertain whether the African haplotypes were present in ancestral human populations and subsequently gave rise to the non-African haplotypes, as concluded by Wainscoat *et al.*, or whether the reverse is more likely.

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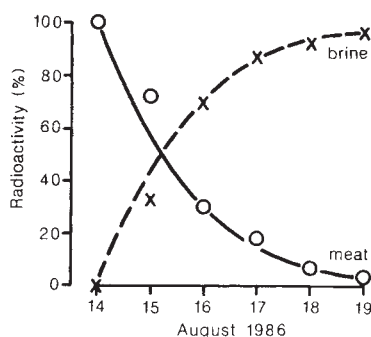


Fig. 1 Extraction of ^{137}Cs from meat by pickling in salt solution. The 100% value for the meat represents 218 Bq per kg. Cumulative values are shown for the brine.

The gift of Taungs

Andrew Hill

Hominid Evolution: Past, Present and Future. Edited by P. V. Tobias.

Alan R. Liss: 1985. Pp. 499. \$38, £38.85.

Major Topics in Primate and Human Evolution. Edited by B. Wood, L. Martin and P. Andrews. Cambridge University Press: 1986. Pp. 364. £19.50, \$39.50.

Primate Evolution and Human Origins. Edited by R. L. Ciochon and J. G. Fleagle. Benjamin/Cummings: 1986. Pp. 396. \$31.95, £29.95.

ON THE face of it, a casual student of scientific methodology would find the basic principles of palaeoanthropology fairly easy to discern. They might be expressed as a few simple rules: every new fossil hominid specimen is the most important ever found and solves all known phylogenetic problems; every new hominid specimen is completely different from all previous ones, no matter how similar; every new hominid specimen is a new species, and probably new genus, and therefore deserves a new name. Although these precepts form a not too exaggerated characterization of procedure in the subject until quite recently, clearly the adoption of them is not likely to create the unambiguous truth about the issues they would address.

Not all claims can be uncritically discarded however, for some discoveries actually happen to be as important as their proud discoverers say they are. Consequently another, but less publicly conspicuous, aspect of palaeoanthropology is the evaluation of new material and the statements about it. Typically extravagant assertions surrounded the discovery in 1924 of a fossil juvenile skull from the Buxton Quarry at Taung in South Africa. Describing it as a human ancestor and "...one of the most significant finds ever made in the history of anthropology...the ultimate answer in the...study of the evolution of man", Raymond Dart named the curious specimen *Australopithecus africanus*. The predominantly British palaeoanthropological establishment, enthralled by Piltdown Man, said the thing was just an ape, nothing to do with the human lineage, and *Nature* hesitated before publishing (115, 195). In fact, Dart turned out to be correct. The Taung baby formed a turning point in the study of human evolution.

The most obvious influence of this change, the subsequent attention on Africa, permeates all three of these books, but quite deliberately in *Hominid Evolution: Past, Present and Future*. Sixty years after the Taung discovery, Phillip Tobias, one much fascinated by anniversaries, organized a jubilee international symposium to celebrate the occasion. This volume is the result. Along with the other two books, it is a forceful and reassuring

demonstration of the extent to which the subject has developed since then.

At least three areas of development seem prominent now, one being perennial. The treasure-hunting element of palaeoanthropology, with the attendant squabbles and hyperbole, may sometimes smack a little of non-science, but new fossils remain vitally important to the subject. They are the source of the biggest surprises and the greatest advances, and they need not be fossils of new species. Much of the anatomy of recognized species is relatively unknown, and fossils that illustrate previously unknown anatomy permit more refined phylogenetic and behavioural assessments to be made. Or they can be already known forms from new time periods or places. The many new discoveries of the past 60 years have resulted in major developments.

Second, progress has also been made, which shows evidence of being more than fashion, in the analysis of these bones. This is largely due to cladistics. There are many who would not pursue committed cladists to the limits of their techniques and inferences; even so, they have adopted Hennigian terminology and principles as a valuable way of minimizing error. At the very least, cladistics forces workers to make explicit their taxonomic ideas, and clearly exposes the implications of different phylogenetic assertions. At this level alone it has made a significant contribution.

A third advance, as far as patterns of taxonomic branching are concerned, and to some extent the dating of them, has been the increasing refinement and acceptance of comparative molecular work on modern primates. Palaeontologists have realized, based largely on new fossils and the cladistic approach, that their ideas of early hominid divergence times were wrong, and that the more recent estimates suggested by molecular comparisons are more plausible.

Hominid Evolution contains over 50 papers which reflect these changes. They range from historical overviews of different aspects of the field, to treatments of particular anatomy of particular fossils and the details of different techniques. In time, the papers extend from the earliest higher primates to the origins of groups of

modern humans. As may be expected of a large volume of such large scope, the result is a little patchy, with some articles admirable and others less so. But overall this is clearly an important publication, essential to all seriously concerned with the subject.

The books are not really to be regarded as alternatives — they have different origins and aims — but on balance I think *Major Topics in Primate and Human Evolution* a more interesting overview than *Hominid Evolution*. This also came from a recent conference, but one intentionally more focused. Delson (*Nature* 313, 532) gives a summary of the presented papers. The subject is the relationships among taxonomic groups and the nature of divergence within the whole of primates. The sense of greater integration received from this book comes partly from this focus, and partly from the organizers' technique of inviting authors to review subjects not their prime speciality. A good example of this is a paper by Peter Andrews, who reviews the molecular evidence in a way that is highly accessible to others outside that field. An excellent article by Robert Martin, discussing the definition of primates, also includes a lucid and terse account of the benefits and limitations of cladistic thinking.

Primate Evolution and Human Origins also covers the whole of the primate order. Ciochon and Fleagle have collected papers on a broad range of issues, extending back over the past 25 years, though most are more current. Their choice was guided not by what is still correct, but by what has been influential, and this makes a very interesting and salutary package. The articles are arranged in groups in a useful way with short introductory essays from the editors. It is valuable to have such classic articles in one easily accessible place, but the book also highlights the direction that thinking, and sometimes misthinking, has taken. It is probably too much to hope that we will learn from our errors, but this latter aspect of the volume assists in the attempt.

Sir Peter Medawar, in a phrase ambiguously directed either to palaeoanthropology in general, or perhaps more understandably to Teilhard de Chardin's practice of it, once spoke of "a comparatively humble and unexact kind of science". Such implied criticism could be justified; it has certainly been possible to get away with being an unexact practitioner. But the aims are far from humble, and as these books well attest, the practice is becoming guided by a set of rules that are more exacting and rigorous than a casual student of scientific methodology might suspect. □

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Together through history

Juliet Clutton-Brock

In the Company of Animals: A Study of Human-Animal Relationships. By James Serpell. Basil Blackwell:1986. Pp.215. £14.95, \$19.95.

"A DOG is for life not just for Christmas."

It is easy to believe that this epigram symbolizes a new concern for the welfare of animals and that this concern is an innovation of the Western world in the present century. Yet the earliest authors of the classical world gave instructions on the care and breeding of domestic animals and modern farmers could benefit from following the advice of the Roman writers Columella and Varro. Since the industrial revolution and the movement of people away from the land into the cities, however, there has been a loss of contact between humans and animals which has been, at least in part, responsible for the attitude that livestock are nothing more than food-producing machines. In his book, *In the Company of Animals*, James Serpell explores this development with a rather disordered but well-documented combination of history, philosophy and

animal behaviour.

There are three interwoven themes to the book. First, the history of the relationships between humans and animals from the hunter-gatherers of the early Holocene to the factory farms of today. Secondly, an analysis of the dichotomy between the economists who look only at the profit to be made from their animals and the pet-owners who will go to any lengths to ensure the well-being of their cat or dog. The third theme is concerned with observations on the behaviour of domestic animals and this is the most tantalizing because, in the burgeoning field of ethology, it is strange how very few studies have been carried out on the natural behaviour of domestic animals. Indeed, it appears not to be generally recognized that domesticates have any natural behavioural patterns. A dog is not looked at, even by an ethologist, as a social hunter but as a companion or nuisance; a cat is seen not as a solitary hunter but as a soft warm creature that requires milk at regular intervals.

The book begins with a summary of the behaviour of the wild boar, a sociable and intelligent animal, and this is followed by a description of the very unpleasant conditions that can prevail with the intensive rearing of its descendant, the modern domestic pig. This is contrasted with the excessive care that may be lavished on pets by their owners.

Serpell touches on almost all the rela-

tionships between humans and animals from ancient bear cults to the slaughter of exotic animals in Roman amphitheatres to present day pet-therapy. He analyses the neuroses of over-indulged dogs and describes the mechanisms by which people distance themselves from the animals they treat badly or kill. There is brief discussion on changing attitudes to anthropomorphism — a dirty word to the clinical scientists of the 1950s, but nowadays acceptable, so that it is again permissible, for example, to describe the mood of a dog as cheerful or jealous. Along with this changing attitude is a realization that humans have associated closely with other species of animals for thousands of years and that personifying them is a normal part of human behaviour. It is not eccentric and it may even be therapeutic.

Many of Serpell's observations are incontrovertible, perhaps because they appear to state the obvious, but they bear repetition because of the very real need to improve the standards of welfare of millions of animals. The founding of the first professorship in animal welfare in Britain (the Colleen Macleod Chair at Cambridge) reflects this need and it will be an added fillip if this book can help to inspire new work into the behaviour of domestic animals. □

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THE MANAGEMENT OF AIDS PATIENTS

Edited by DAVID MILLER, JONATHAN WEBER and JOHN GREEN

The Management of AIDS Patients is the first comprehensive guide to the practical clinical management of patients with AIDS or HTLV III infections. The book avoids the sensational aspects of the disease, offering solid advice and information for all people involved in patient care.

The editors and many of the contributors come from St Mary's Hospital, London one of the foremost centres in Britain for the treatment of AIDS patients. The knowledge and experience of these experts has been combined to provide a much-needed book for doctors, nurses, dentists and indeed for health-care professionals everywhere.

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Stellar challenge

Eugene N. Parker

Physics of the Sun. Edited by P.A. Sturrock, T.E. Holzer, D.M. Mihalas and R.K. Ulrich. Reidel:1986. Vol. I *The Solar Interior*, pp.257, £36.25, \$44.50. Vol. II *The Solar Atmosphere*, pp.385, £49.95, \$62. Vol. III *Astrophysics and Solar-Terrestrial Relations*, pp.287, £38.95, \$49. Set £99.75, \$125.

IN RECENT years the physics of ordinary stars has developed into a flourishing subject. The Sun is the standard bearer — the only star that appears as more than a blur of light in the telescope. Close examination shows it to be a remarkable object, with a complicated personality and its own private secrets.

Outwardly the Sun shows enormously variable emission in the radio range and in ultraviolet rays, x-rays, γ -rays and cosmic rays. The source of these radiations is the active outer suprathermal atmosphere, which is produced largely by the magnetic fields that erupt through the surface in a remarkable fibril form. The activity of the Sun is driven by the fibrils, whose structure and motion is on so small

a scale that they can be observed only by a large telescope in space. The Solar Optical Telescope was designed to provide those essential observations, and when, and if, such an instrument is launched into space we should then be able to examine the inner workings of stellar activity.

Essentially all stars show variable x-ray emission, indicating that they are as complex as the Sun, and some much more so — but it is difficult to make much of their wide range of activity until the physics of the Sun is firmly in hand.

The observation of neutrinos from the Sun has established that there is something missing or mistaken in present theory of stellar interiors. The theoretical construction of the stellar interior is based on a number of reasonable assumptions, but it has failed to predict the observed neutrino emission by a factor of four or five. The difficulty may lie in a finite rest mass of the neutrino, with profound implications for both particle physics and cosmology. Or it may lie in an erroneous estimate of the helium content or in the theoretical opacity, or in the laboratory value for a nuclear reaction rate. It may, therefore, have a serious effect on our theoretical understanding of all stars. Fortunately, forthcoming observations of low-energy neutrinos and the new subject

of helioseismology together should go a long way towards clearing up these questions.

In view of all this, the three volumes of *The Physics of the Sun* have made a timely appearance, in an appropriate form for the physicist. Not only do the 31 authors of the 22 chapters review the present state of knowledge, but they press on to consider the outstanding puzzles and dilemmas, summarized in the introductory chapter. For that reason the books will serve as a basic reference source for the next decade, and perhaps beyond.

It will be interesting to look back from the anticipated wisdom of the 1990s to see if the resolution of the neutrino discrepancy lies in an error in the thermonuclear reaction rates (reviewed in Chapter 2), or in the radiative transport in solar matter (Chapter 3) or perhaps in some unexpected mixing in the central regions (Chapter 4), which may in turn be related to the turbulent circulation in the convective zone (Chapter 5) and internal stresses (Chapter 6). In all probability, however, these questions cannot be resolved without an understanding of the structure of the solar interior to be derived from solar oscillations in the manner described in Chapter 7.

Essentially, all information concerning the activity and composition of the Sun comes from observations of the solar atmosphere, reviewed succinctly in Chapter 8. The active suprathermal atmosphere is described in Chapters 9 and 10, and the dynamics of magnetic fields and fluids responsible for the activity in Chapters 11 and 12. The acceleration of fast particles and the associated nuclear reactions in flares are reviewed in Chapters 13 and 14, with the radio emission from the many forms of outburst treated in Chapter 15.

Chapter 16 reviews present facts and ideas about the formation of the Sun and planets. Chapter 17 provides a critical discussion of the problem posed by the low neutrino flux, with an examination of some of the possible causes. Chapter 18 treats the solar wind phenomenon in the broad context of all stars, with coronae and winds. The magnetic activity that drives the suprathermal atmosphere of the Sun and other stars is covered in Chapter 19, while the last three contributions review the effects of the hard radiation, the solar wind and the fast particles on the terrestrial environment.

There has been no more vivid portrayal of the diverse and exotic phenomena that make up a "pedestrian" star like the Sun. One needs only to be close enough to see the details to discover, and to be challenged by, the physics of our nearest star. □

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Picture histology

Nancy J. Lane

A Colour Atlas of Insect Tissues via the Flea. By Miriam Rothschild, Yosef Schlein and Susumo Ito. *Wolfe Science, London/Methuen, New York: 1986. Pp. 184. £40, \$60.*

THIS large, well-bound volume is intended to fill the gap between two earlier books, one on the general anatomy of insect tissues and the other on the fine structure of individual insect cells. It contains numerous colour micrographs of sectioned histological preparations from the various organs of the flea; together with higher resolution electron micrographs of many of the same tissues, these splendid plates make up the bulk of the book. A series of beautiful line drawings preface the plates and illustrate the flea's principal internal organs. As a guide to the histology of insect tissues this is an admirable compendium since it deals with all the component organs, and for each there is a separate foreword describing its organization and function.

In their introductory remarks, the authors express the hope that the colour micrographs will "stir the imagination and excite the aesthetic susceptibilities" of students. This they almost certainly will do, as may the cover, a particularly striking multi-coloured histological section of a gravid flea's oviduct and vagina!

The rationale for the *Atlas* is to illustrate the cellular organization of the tissues of all insects, using the flea as a typical example. The choice of organism was appropriate because the flea's small size allows the cutting and mounting of sections through the entire animal, so that many organs can be observed at once. Moreover, as an ectoparasite and potential carrier of bubonic plague, myxomato-

sis and typhus, the flea is a good example of a vector of arthropod-borne diseases of mammals. As such it has certain peculiarities, and at the same time lacks other features, but an appendix illustrates some of these latter structures from other insects.

There are a few inaccuracies — such as the mislabelling of what is probably a gap junction as a tight junction (these are only present in certain tissues in insects), and in the definition of "perineurium" (which consists of the glial cells but *not* the connective tissue surrounding nerves and ganglia) — and a degree of repetition in some illustrations; at times, one finds oneself wishing that the second of two rather similar micrographs was at a higher power to resolve more detail. Some tissues are heavily illustrated, whereas others, for example the dorsal vessel, have only one accompanying light micrograph. There is an inconsistency, too, as to whether or not (or indeed how many) electron micrographs of each tissue are provided; some, such as the sensillum and the unfortunate dorsal vessel, have none! A few more specialized references would also have been welcome for those not so well-versed in the subject as the authors.

The book will undoubtedly be extremely valuable to the novice, and will also serve as a practical guide for established entomologists wishing to examine organs with which they are unfamiliar; conveniently, it is bound so as to remain flat when opened for study. It is likely to be widely used and a copy ought to be available in every department that actively pursues insect physiology. That it also has the glossy appearance of a coffee-table text need not detract from its usefulness and practicality in the laboratory. □

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All steamed up — geyser activity near Healdsburg, California, 100 km north-west of San Francisco. The picture is reproduced from *The Geology of Ore Deposits* by John M. Guilbert and Charles F. Park, Jr, published earlier this year by W.H. Freeman.

Physics and Homi Bhabha

Rudolf Peierls

Homi Jehangir Bhabha: Collected Scientific Papers. Edited by B. V. Sreekantan, Virendra Singh and B.M. Udgaonkar. *Tata Institute, Homi Bhabha Road, Colaba, Bombay 400 005, India:1986. Pp.1,023. Institutions Rs 500; individuals Rs 250. Outside India \$150.*

HOMI Bhabha was a highly talented scientist, and also a painter of distinction. But he will be remembered above all for the part he played in the development of science and atomic energy in India.

Bhabha came from a well-to-do and sophisticated Parsee family. After studying mechanical sciences at Cambridge, he started research in theoretical physics in 1930. This was an exciting time for physicists and Bhabha became attracted by the phenomenon of cosmic rays. He wrote his first paper on this subject, which remained his dominant interest thereafter. He developed the theory of cascade showers (simultaneously with Carlson and Oppenheimer) which explained many of the otherwise mysterious observations, and led to the conclusion that the penetrating component could be explained only by a breakdown of the quantum theory, or by a new particle, which indeed turned out to be the muon. His many papers on other subjects, such as electron-positron collisions ("Bhabha scattering"), new kinds of wave equations, and meson theory, all had potential applications to cosmic rays, which he kept clearly in mind.

By the late 1930s Bhabha was well established in Cambridge, while also visiting many other European institutions. We do not know how long he would have continued in Britain, but he happened to be in India on holiday when the Second World War broke out and prevented his return. He became a Reader, and later a Professor at the Indian Institute of Science in Bangalore, and built up a strong group, mainly on cosmic-ray research.

Bhabha, however, was also endowed with formidable administrative energies. He saw the need for a national institute devoted to fundamental research, and on his initiative the Tata Institute was founded, with him as director. It became an institution of high standard and exerted a profound influence on science in India. Later he realized the importance for India of nuclear power and persuaded the government to set up the Atomic Energy Establishment in Bombay, of which he was put in charge. He also became the chairman of the Indian Atomic Energy Commission. As he was also chairman of the Scientific Advisory Committee to the

Cabinet, devotees of Gilbert and Sullivan might well have thought of him as the "Poo-Bhabha"! But he managed to carry out all these functions efficiently.

He did not, however, regard science from a narrow, national point of view — he was also concerned with the international relations between scientists. For three years he was president of the International Union of Pure and Applied Physics, he chaired the Geneva Conference on the Peaceful Uses of Atomic Energy in 1955 and also served in the International Atomic Energy Agency. Bhabha's death at the age of 56 in a plane crash on Mont Blanc was thus a sad blow not only to India, but also to international science.

On the 75th anniversary of his birth the Tata Institute has published this commemorative volume, most of which is taken up by his scientific publications. Some of these are familiar today, because they have become part of our recognized knowledge of physics, while others were exploring new paths which the development of physics did not follow. The collec-

tion is therefore not likely to become a research tool, but it is of great interest to the historians of physics, and to readers who want to appreciate the work of a remarkable man. Only his strictly scientific papers are included, and one hopes that Bhabha's speeches and writings on science policy and other general matters will be published separately.

Three introductory articles by B.V. Sreekantan, Virendra Singh and B.M. Udgaonkar outline Bhabha's work in cosmic-ray physics, general theory and science policy. Tributes to Bhabha at a memorial service by Cockcroft and M.G.K. Menon, and Penney's Biographical Memoir, are added as appendices.

I would have liked to see inclusion in this attractively presented book of reproductions of one or two examples of Bhabha's paintings. There is only a drawing of Blackett, hardly typical of Bhabha's work. □

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Scanning standard

V.E. Cosslett

Scanning Electron Microscopy: Physics of Image Formation and Microanalysis. By Ludwig Reimer. *Springer-Verlag:1986. Pp.457. DM112.*

IN HIS preface to *Transmission Electron Microscopy* (Springer Monographs in Optical Sciences No.36), Ludwig Reimer stated that the scanning method would be covered in a complementary volume. He has now fulfilled this promise some two years later. In the present book (No. 45 in the same series), he uses the same approach as in the previous volume (*Nature* 309, 186). But perhaps this is not surprising, as both books evolved from lectures given at the University of Münster. Translated into very readable English, with the aid of the guest editor, Dr P.W. Hawkes, *Scanning Electron Microscopy* matches the standard of its predecessor to give an authoritative and up-to-date account of the subject.

Most aspects of the subject are dealt with, except for the applications of the scanning system, which were included in an earlier text of Reimer's (*Elektronenmikroskopische Untersuchungs- und Präparations-Methoden*), and are to be found in several congress reports that have since appeared. Special attention is here given to the physical basis of scanning microscopy. The detailed processes involved in beam-specimen interaction and in image formation are first considered. Naturally there is a degree of overlap with the previous volume in respect of the

former, but rather less emphasis is placed on the wave nature of the electron. A quantum mechanical treatment is given of elastic scattering and inner shell ionization, but multiple scattering and (in thicker specimens) diffusion enter frequently into scanning microscopy, and here the electron appears as a particle, especially in secondary and backscattered imaging. The simple concepts of mean free path and mean energy loss are consistently invoked. The consequences of the Bethe and other models are displayed, and compared with experiment. The emission of X-rays is fully discussed, in relation to microanalysis and to some extent with diffraction. There is a good exposition of the theory of electron diffraction and its part in channelling patterns.

Other chapters deal with cathodoluminescence, electron beam induced current, detectors and signal processing, but there is no detailed description of apparatus for microscopy or microanalysis. The author's aim is to make clear what happens inside them, and especially in image formation and interpretation. As he says in the preface: "Many SEM users do not give much thought to the origin of contrast, but when the signals are to be used more quantitatively it becomes necessary to know more about the physics of SEM". In this aim he has succeeded admirably, and his book is likely to become the standard work for all users of the instrument. Especially to be commended is the list of references (running to 140 pages) which closes it. □

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National security and the post-war science establishment in the United States

from A. Hunter Dupree

Since 1940 science policy in the United States has been set within the framework of an unwritten constitution that allows flexible responses to be made through advice from the scientific community. Recent developments under the Reagan administration suggest this system is foundering.

Government support of science in the United States falls into two great periods—before and after 1940. The wartime research programmes and the first nuclear explosions in 1945 were the result of great changes in research organization, not their cause.

In the nineteenth century, support of science by the national government of the United States had come largely through the Department of Agriculture, the Geological Survey and the army and navy. Most of it went to government employees working in the life and earth sciences, for applied research closely related to government. After 1900, support increased modestly, and newer agencies—the Weather Bureau, the National Bureau of Standards and the National Advisory Committee for Aeronautics—began to incorporate scientists in the universities, especially those in the physical sciences.

Vannevar Bush, an electrical engineer from the Massachusetts Institute of Technology, who in 1938 had moved to Washington to head a major research foundation, was on hand to undertake the organization of research for war for President Franklin D. Roosevelt in the dark days of June 1940. By 1941 he was Director of the Office of Scientific Research and Development (OSRD). From the beginning through to the post-war world he played a central role in revolutionising the pattern of patronage and institutional structure of science.



Vannevar Bush, the visionary engineer.

Bush also set the mould for the interpretation of the relation of science and government in the post-war United States to which most students of science policy and historians of science have subscribed, especially in their attempts to evaluate the relation of the military to science. The clear break in history seen in 1940 was the creation of a special committee of scientists from outside the regular government service who would direct research on weapons for immediate use in the war. The research sponsored by the scientists was seen, in the nuclear explosions of 1945, to have created powers that would force military strategy to be recast.

Bush tradition

The organization created by Bush in 1940 broke with the science establishment already in place in government laboratories. OSRD fashioned a contract by which laboratories in industry or, more often, in universities were given large sums of money to perform specific research on a project related to weapons that promise a payoff in the then-present war.

The members of the panels of the OSRD who designed the contracts and allocated resources were themselves scientists, some of whom, like James B. Conant of Harvard and Karl T. Compton of MIT, were also university presidents who could use their pre-war recruiting networks to identify key personnel. Each project was steered by a scientist, the principal investigator, who chose his team of associates on the basis of scientific merit, often turning to his own recent graduate students.

In theory, Bush and his panels could refuse to work on a project wanted by the military and, equally important, they could insist on pushing a line of research that the services did not want. The sole point of contact of OSRD with the US government was through Vannevar Bush's personal relation with the President, Franklin D. Roosevelt. The only link with Congress was an occasional background briefing of selected members of the appropriations committees, who passed the money under vague descriptions scattered through the budget. The existence of OSRD was public knowledge through

announcements in places such as *Science* magazine, but both the public relations of the organization and Congressional liaison were handled by Bush himself in what little spare time he had.

As well as refusing to take general responsibility for all weapons research, Bush also refused to take responsibility for the upgrading of industrial research on behalf of the war effort, as at least one member of the War Production Board had wanted him to do. Thus OSRD research was targetted, and both the aim and the conduct of the research in non-government laboratories were controlled by scientists. Although much of the research was done in universities, OSRD was not responsible for the training of research personnel or for basic research for its own sake. Whenever a university scientist tried to smuggle his favourite long-term basic research into a weapons project, he was promptly visited, often by Conant, and redirected.

Post-war research strategy

Bush's awareness of the emergency nature of OSRD is reflected in his post-war plans. The report, *Science, the Endless Frontier*, was produced in 1944 to bring about the speedy release of the results of OSRD research into the public sector, to close the four-year gap in graduate education by federally funded fellowships (although direct federal support for undergraduate education, even in the sciences, was then too much to contemplate) to support medical research in the same way as weapons research, continuing the OSRD practice, whether the medical professions liked it or not; and to create a National Research Foundation that would perpetuate the OSRD research contract as a funnel for federal funds into fully independent universities and industry.

The targetting of federal support for basic research was to be controlled by scientists in the foundation, which would answer only to a part-time board of citizens. The foundation would make OSRD-type contracts for research projects with universities, where the actual work would be in the hands of the principal investigators who, as professors, would

owe their loyalty to the scientific community and to their university and not to any administrative superior within the government.

The plan put forward in *Science, the Endless Frontier* has won fame as the blueprint for the institutions that now exist within the US government, specifically the granting agencies that support scientific research. Short histories of government and science after the Second World War are often no more than histories of the National Science Foundation, established in 1950, and the National Institutes of Health, which took over the OSRD contracts in medicine immediately after 1945. Since the armed services emerged from the war with the conviction that basic research was necessary to their missions, they established agencies of their own which supported basic research in the universities, beginning with the Office of Naval Research in 1946 and followed later by the Air Force Office of Scientific Research and the Army Research Office.

Matching the granting agencies within the government were the universities which had the strong faculties, good laboratory facilities and strong accounting and business staffs necessary to handle the unprecedented flow of money from the federal government. The research universities, some fifteen or twenty in the immediate post-war years, both generated the proposals and sent to Washington their most distinguished professors to sit on the panels that judged the proposals' worth.

When the system worked well, federal funds flowed to the disciplines of science in the universities in total disregard of technological applications or political influence. The results flowed out into the open literature, the research received undreamed-of support without seeming to suffer constraint by government and the prestige of science assured the government and its taxpayers that financial accountability was almost automatic. When spokesmen for the scientific community speak of the Report as their *credo*, they are thinking of the combination of support and freedom from restraint that has flowed from this system.

Post-war weapons research

Bush himself always insisted that OSRD should be closed at the end of the war and, in spite of opposition, he had his way. There were good reasons for his stand—the lack of public and Congressional support and the impossibility of recruiting good scientists under post-war conditions. The problem left unsolved by the demise of OSRD was where, in Bush's post-war system, would weapons research fit.

Alex Roland has summed up Bush's position as this: war is too important to be left to the generals; and science is too important not to be left to the scientist¹. Bush's early efforts to solve the problem



Scientists at the White House. Left to right: James Killian Jr, George Kistiakowsky, Edward David Jr and Frank Press.

failed. A Division of National Defense in the proposed National Research Foundation came to nothing; when the National Science Foundation finally came into existence in 1950, the division of National Defense persisted as a dormant vestige of past intentions. Another Bush effort, the Research Board for National Security of 1945 and 1946, foundered in part because the Bureau of the Budget, in the Executive Office of the President, would not funnel huge funds to beyond the control of the President and into the National Academy of Sciences.

It is easy to gloss over these stillborn efforts and to say that the Office of Naval Research, with its OSRD-type contract relations with universities in support of basic research and, from 1946, with its own statutory authority, was the successor to the OSRD in weapons research. The Bush-oriented account of military research, however, does not acknowledge one very obvious feature of research during the Second World War—that most US military research was actually done either within the technical bureaux of the services or in the operating units themselves.

A knowledge of pre-1940 relations between science and government would have led one to predict that the mass of weapons research close to the missions of the armed services would have been kept within their control, with scientists brought into the tight circle of security classification only at the behest of the services. The technology of war in the twentieth century, like that in any other century, is a congeries of craft traditions oriented to age-old military cultures, which are in turn enmeshed in culture.

The peculiar prominence and power of science in the Second World War came from a very few critical components, emanating from research laboratories, that dramatically transformed the weapons available to the services and the systems in which they were used. OSRD, through such dramatic developments as the family of radars at the Radiation Laboratory at MIT or the uranium and plutonium bombs assembled at Los Alamos (the Manhattan Project was an offshoot of OSRD), transformed the system. Then and since, the services have both maintained and

developed the technology with which one of the great wars of history was fought and have also redesigned those systems as the components supplied by scientific research became available.

While Bush for a short time, in the period 1946–48, may have had dreams (not to say delusions) of controlling military technology through boards of civilians, mostly scientists, acting autonomously and unchecked by the political system, he had by 1948 largely withdrawn from this effort, leaving the Research and Development Board of the newly created Department of Defense to suffer a slow decline. More enduring was the Bush legacy of providing scientific advice to the President directly, independently of control by the services or the Department of Defense.

Eisenhower and atoms

The decision to achieve nuclear fission and to make a bomb—an exploding device that would touch off an uncontrolled chain reaction—was made by scientists working within the Bush framework. First in OSRD itself, then in the Manhattan project, and finally in the post-war Atomic Energy Commission, scientists had a major voice in the choices that built the most powerful component of a weapons system in all military history. They made those choices in almost total disregard of the environment in which their component would be detonated. In the tradition of the scientific laboratory, they gave careful attention to the safety of the experimenters who built the bomb, but by sealing them off from society, they addressed neither the consequences to the general public of an accident with the new technology nor the consequences to the world and its inhabitants of a war fought with such uncontrolled explosions. The battlefield, the theatre of war and the whole world became one, almost from the first detonation in New Mexico.

In the 1950s, the US Atomic Energy Commission (AEC) presided over research in nuclear physics and the making of nuclear weapons, largely in national laboratories, and was also the monopoly manufacturer of fissionable materials. Dwight D. Eisenhower has recently been portrayed as a president who understood

the horrors of nuclear war and hoped to compensate for them by reaping the benefits of nuclear energy for peaceful purposes². That balance required control of chain reactions. Control has a physical meaning when a chain reaction runs uncontrolled into an explosion. Control has a political and diplomatic meaning when one speaks of international control, for example by limiting the proliferation of nuclear weapons. Control could be achieved only by technological systems, which in turn were part of the political, diplomatic, and economic systems of the nation states that took on the responsibility of possessing nuclear reactions in any form.

The Bush ideal of having scientists in control by the agency of boards insulated from politics was echoed in the structure of the AEC, but by 1950 President Harry S. Truman's decision to proceed with the hydrogen bomb had clearly signalled that the scientists, even with their stellar General Advisory Committee, were unequal to the task of making policy for what had become nuclear technology. The Oppenheimer case, which concerned policy judgment as well as loyalty and security, put the ambivalence of scientists, as the would-be controllers of the forces they had unleashed and as the servants of the power of the nation states, on stage as a tragedy for all the world to see.

The expanding debate

Eisenhower put his hopes in the Atoms for Peace programme, and persevered with it, with considerable accomplishment, in spite of the challenges of doubtful economics (except perhaps in Western Europe), the threat of proliferation and the difficulty of negotiating international cooperation. The pursuit of the peaceful use of atomic energy provided a justification for continuing the pursuit by the United States of supremacy in nuclear science and technology. But the peaceful uses of the atom were never sufficient to relieve either nuclear scientists or the US government of the dilemmas with which the advent of the new weapons had enmeshed them.

Indeed, the 1950s marked the demise of Bush's ideal of scientists as the directors of the whole of nuclear technology. Only over the building of research reactors probing particle physics did they retain a modicum of control. Power reactors were designed first for the US Navy to provide propulsion for ships and then as components in conventional electric power generation systems, merely another source of heat. Even by 1960, it was possible to glimpse the later troubles of the nuclear power industry—the difficulties of peaceful uses internationally, the dissolution of AEC and the military's overwhelming commitment to a nuclear strategy.

The broadening of the nuclear debate

to include the public began under President Eisenhower, who is now seen as encouraging the trend. But changes did not come without struggle for the many groups, including scientists, who joined the controversy uninvited by the establishment³.

The evolution of the nuclear issue from one of science to one of technology, for both war and peace, provides part of the background for Eisenhower's classic outburst against the military-industrial complex, in his farewell address in 1961. Policy was being made not by scientists based in universities but by the military establishment and their industrial contractors.

The president had to issue a clarification to his science adviser, George Kistiakowsky, one of those who had built the bomb but who, to the end of his life, identified with the free scientific community and the American democracy to which he had come from Russia. Eisenhower's major point, according to Kistiakowsky, "was his conviction that the part of science which is engaged for armaments purposes must never be allowed to dominate all of science or curtail basic research"⁴.

National defence in space

Many features of the conventional account of American participation in the arrival of the space age in the late 1950s can be described wholly in the terms of the Bush tradition. The International Geophysical Year, an international organization in the hands of scientists, was to put up the first Earth-orbiting satellite as a scientific experiment. US participation was controlled by a committee of the National Academy of Sciences, and the appropriation authorized by Congress came via the National Science Foundation. In spite of all the public criticism when the Soviet Union, with Sputnik I, beat the Vanguard satellite into orbit, the American scientists insisted that in the end they had produced genuine and even spectacular results.

The civil, open and international character of the first effort was reflected in the steps taken in the wake of the Soviet triumph. President Eisenhower appointed a science adviser and gave the President's Science Advisory Committee direct access to himself. The National Aeronautics and Space Administration (NASA), created in 1958, departed from the Bush pattern by having a single administrator rather than a multiple executive, and its responsibility to the public and to Congress made it political in a very broad sense. Although dependent on propulsion developed by the military, NASA was nevertheless clearly separated both from the armed services and from the Department of Defense. Indeed, in looking into the "missile mess", President Eisenhower had a tendency to use his

scientific advisers as a counterweight to the military influence in his administration.

The role of the military services in the development of American ability to enter space has, of course, been implicitly acknowledged from the beginning. But, in delineating the development of a space mission within the military, historians have pointed to a very different group of scientists and a very different group of research institutions from those associated with the atomic bomb or the other programmes emanating from OSRD. By the 1950s, the interest of the US military and intelligence services in reconnaissance over the Soviet Union gave a strategic reason for American entry into space⁵. Those who look at the military origins of space research argue that entry into space came not from the scientific institutions in the Bush tradition, but from the conjunction of two major systems that flowed together just after the Second World War.

The race into space

The two systems came out of the military services, and neither of them had anything to do with nuclear energy. The rocketry which made possible the velocities needed to escape into space was largely developed in Germany during the 1940–45 war, and dispersal of German rocket experts both eastward and westward provided the vehicles for the Soviet and American space programmes. In the United States, these vehicles were developed almost entirely within the military services by teams which included many of the German experts, some of whom, like Wernher von Braun, eventually rose to high administrative positions.

The other ingredient of success was the network of command posts, control systems, communications systems and sensors for tracking objects which had its beginnings in the radar and radio control of fighter planes in the Battle of Britain. Late in the Pacific war, the US admirals in command of amphibious landings flew their flags not on battleships but on unarmed command ships loaded with electronic gear.

Only now, in the 1980s, has the command, control, communication and intelligence system for nuclear war been given publicity under a single name, C³I (ref. 6). The components had been developed during the Second World War, some of them in the laboratories of OSRD. But it is the services themselves that have put these electronic components together into fire-control systems, fighter director systems, communication systems, navigational systems and their many variants in the recognizable precursors of C³I.

By the mid-1950s, the two elements, the electronic envelope of C³I and the rockets capable of ever-increasing lift and velocity, came together with the nuclear

warheads to produce the intercontinental ballistic missile. The Cold War, the strategy of mutual deterrence, the whole of the space program, even the development of the digital computer, took on their characteristic shape in the light of these developments.

The "I" in C³I stands for intelligence; the extension of the electronic envelope so that sensors can report on Soviet territory itself is completely in line with the technical thrust of reconnaissance research within the services ever since the Second World War. Only more recently, with the use of the space shuttle to launch a military satellite, has reconnaissance come into public consciousness in the United States⁷.

The joining of rockets and the electronic envelope to a third partner, the nuclear warhead, has made it difficult to achieve a space programme uninfluenced by military considerations and questions of national prestige. Priorities could not be left to the natural flow of proposals from an academic community not attuned to the problems and challenge of spaceflight.

Even so, the Bush tradition of scientific dominance in making policy for military research and another aspect of that tradition, that of the scientist as adviser to Presidents, found a major opportunity in the space age and its military applications. Bush had played such a role to Franklin D. Roosevelt. In 1950, an adviser to President Truman, William Golden, saw the need for a President's Science Advisory Committee even while the structure of the National Science Foundation was being shaped⁸. The flight of *Sputnik* brought science into the White House when James R. Killian and George Kistiakowsky served successively as advisors to President Eisenhower, and a broadly representative President's Science Advisory Committee gave the scientific community a voice even in the most sensitive military issues and a place close to the president.

Changes in policy priorities

The mechanism so laboriously constructed for scientific advice to the president lasted only a little over a decade from the time of Eisenhower's farewell address. From 1962 on, with the creation of the Office of Science and Technology in the Executive Office of the President, it was technology policy, not science policy, that became prominent, with all that implied for economic, political, diplomatic and military influence that Bush hoped to avoid by the insulation of his system.

After the abolition of the President's Science Advisory Committee by President Richard M. Nixon, science has lost even its titular position close to presidential power. True, President Reagan for a time had a science adviser, but George A. Keyworth had before coming to Washington spent most of his career in a weapons

laboratory and emerged not as a spokesman for the scientific community within the administration but as an advocate of the President's Strategic Defense Initiative. There is no widely representative science advisory committee, and many of those who advised Keyworth, including Edward Teller, came from either Los Alamos or Livermore, the two principal weapons laboratories of the US government. These two institutions had the appearance of being a part of the Bush tradition because of a formal connection with the Regents of the University of California, but the connection with the government-university partnership is an illusion; Teller and the Livermore scientists have had their own channels directly to Washington since at least the 1950s.

Conclusion

The situation in early 1986 is that the government-university partnership set up under the umbrella of the Bush Report is still in existence, but with a static level of support and almost no representation for the scientific community in the making of policy at a high level.

The decision to fund the Strategic Defense Initiative on a vast scale was made without peer review, and no advice from scientists was sought or received. The whole process of research and development was aborted, with the technical choices being announced by President Reagan in advance⁹. The American and British publics had to start paying the bill, and the scarce human resources were commandeered, without the slightest review or evaluation by the scientific community as to whether the project should be undertaken at all. This dangerous departure from post-war practice is unique.

The National Science Foundation and the National Institutes of Health still function within the framework of the post-war system, and those disciplines in the universities that fall within their scope continue to send in proposals and receive support. Yet the Department of Defense is becoming increasingly predominant even in the support of basic research¹⁰. A central scientific organization is scarcely functioning at all.

The story of science and national security in the United States from Bush's *Science, the Endless Frontier* to the present

shows that the whole science policy structure has been a part of the unwritten constitution which covers so many fundamental relations in the American government¹¹. Clearly that policy structure is in disarray whether one looks at the advisory structure in the Executive Office of the President or whether one looks at the post of the Administrator of NASA, under a cloud as a shuttle goes to its doom. Even if one can assume that grant proposals from the universities are still flowing to Washington and that this determines the flow of money at some level, there is no assurance that the usual checks of peer review and open publication are still in good order, especially with funds from the Strategic Defense Initiative. At a higher level, in the making of choices as to the overall direction of research, much evidence points to serious malfunctioning. If repairs are being made they are not evident now.

Is there any hope of change in this bleak picture? Last year, William Carey, the executive officer of the American Association for the Advancement of Science and one of the creative architects of the post-war system, described the present situation¹²:

"Ours is a system of interconnectedness rather than cohesion, but it admits light, ventilation, and improvisation. It prospers through flexibility, excellence in management, risk-taking, and good luck. Choices are considered, and directions readjusted or rejected at hundreds of nodes throughout the public, proprietary, and academic systems; decisions are reached for a multitude of objectives that may, or may not, bear upon the economic or national security goals of transient administrations."

The flexibility and creativity that Carey sees in America's unwritten constitution of science policy has indeed produced adjustments, cut down dominant agencies that threaten to distort the scientific enterprise, and disposed (usually not without difficulty) of costly efforts that have strong political support but insufficient scientific merit. The historian can hope from looking at the past that the potentiality exists for reassembling an adequate policy structure even if it is invisible in the present disarray. □

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Role of the cerebellum in the visual guidance of movement

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Mathematicians, control engineers and information technologists are beginning to take a greater interest in neuroscience. They are perhaps starting to realize that they may be able to learn a few tricks from nature with which to improve their machines. At the same time there is a good chance that neuroscientists will benefit from their input of fresh ideas and techniques with which to attack the problems of understanding neural processing. One area of the brain which seems particularly promising in these respects is the cerebellum.

It is still not known exactly what the cerebellum contributes to the control of movement more than 150 years after Flourens first showed that accurate and co-ordinated movements cannot be made without it. By the early twentieth century Cajal had revealed the beautiful geometrical simplicity and striking uniformity of the cerebellar cortex, together with the contrast between the mossy- and climbing-fibre terminals that it receives from other parts of the brain (Fig. 1). But even he was doubtful as to its function. The seductive simplicity of the structure of the cerebellar cortex has attracted much theorizing: in the early nineteenth century the cerebellum was thought to be a voltaic battery; in the early 1900s it became a proprioceptive telephone exchange; in the 1950s it was a collection of transistor delay lines; and today it is a computer. But the cynic's view is that the usefulness of these theories varies inversely with their content of technological jargon, for despite engineering terminology our understanding of the function of the cerebellum has hardly advanced beyond that of Gordon Holmes in 1917. His description of the loss of movement accuracy and co-ordination suffered by soldiers in the First World War as a result of cerebellar injuries remains unsurpassed. Moreover, what little progress has been made in understanding the cerebellum owes very little to physics and engineering, but much to the introduction of powerful new techniques in neuroanatomy. These have clarified relations between the cerebellum and the rest of the brain, but unfortunately they have still not led to a clear understanding of what the cerebellum actually does. Here I shall take the example of visual guidance of limb movements to show how modern neuroanatomical methods have begun to sketch ways in which the cerebellum helps to guide and co-ordinate movements.

Visuomotor connections

Neuroanatomically speaking, the problem of how vision guides voluntary movements can be reduced to the question of which connections link the visual and motor cortical areas. Until recently this was not considered to be a problem. Many intracortical and interhemispheric (commissural) association fibres connect both neighbouring and distant cortical regions, so it was assumed that the main links between vision and movement were direct neural connections between the occipital and frontal cortex.

But no direct pathways between visual and motor cortices have been found¹. If transmission takes place wholly within the cerebral cortex, visual signals would have to travel via a lengthy series of relays in several cortical areas. Such a precarious route, interrupted by so many relays, seems an unlikely way to organize a system that often has to guide movements extremely rapidly in response to visual signals. It is more likely that the preponderance of short-association fibres in the cerebral cortex, mainly linking contiguous areas, is not designed for motor control at all, but rather to relate efficiently sensory stimuli from different

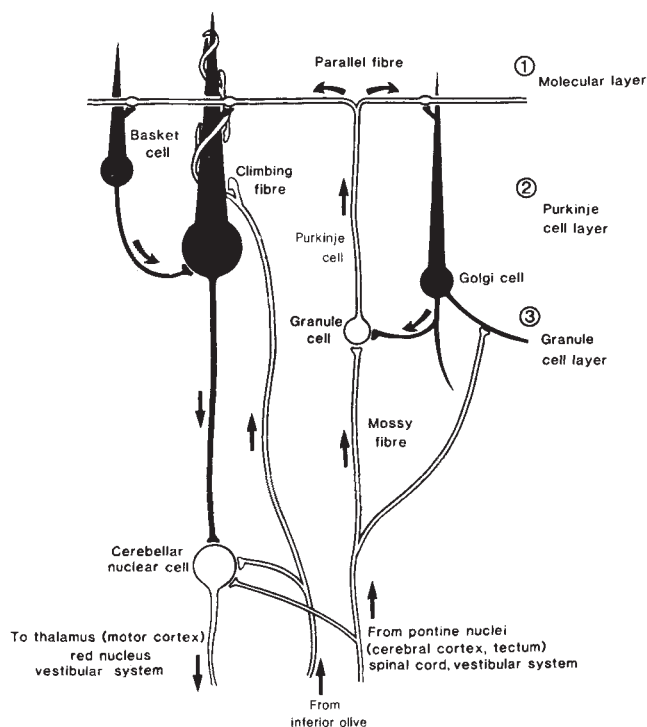


Fig. 1 Mossy fibres carry most of the input to the cerebellum via 10^{11} granule cells and their parallel fibre axons, each of which makes one synapse with many Purkinje cells. Climbing fibres make many synapses with a single Purkinje cell. Inhibitory neurones in black.

sites which are presented at different times, to build up a complete and reliable perception of the external world².

In the 1960s Roger Sperry and colleagues began to obtain the experimental evidence that confirmed that association fibres in the cortex are not responsible for bringing visual signals to the motor cortex. They severed the association fibres underlying the parietal lobe in monkeys, thus disconnecting all the fibres which link the occipital with the frontal lobes, and showed that the animals could still pick up food accurately under visual control, even from a moving turntable³. Moreover, cutting the association fibres in the corpus callosum that join the cerebral hemispheres does not render the arm blind in the contralateral visual field, as would be expected if each motor cortex had to rely on these fibres for visual information about the opposite hemifield^{4,5}. Even removal of the main target of the occipitoparietal visual association fibres, the pericruciate cortex of the frontal lobe, does not prevent monkeys from guiding the contralateral finger, hand or arm by visual control⁶.

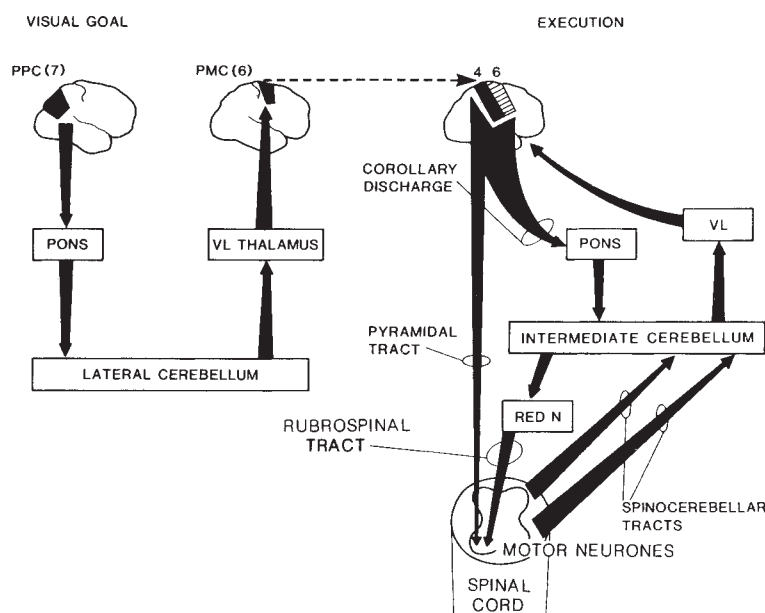


Fig. 2 The connections that link PPC area 7 (Hughlings Jackson's highest level) to the lateral cerebellum and to the PMC area 6 and motor cortex area 4 (his middle level). Areas 4 and 6 then project to the intermediate cerebellum, and from there either back to the motor cortex or to the spinal cord via the red nucleus.

These observations demonstrate that the main routes by which visual information reaches the motor cortex do not run through the cerebral cortex but pass via subcortical structures, probably the basal ganglia or the cerebellum. Both receive visual projections, although there are many more to the cerebellum, and both return projections to the motor cortex.

It is unlikely that the basal ganglia play a significant part in the visual guidance of voluntary movements. They receive only sparse visual projections, and project mainly to premotor, rather than motor, cortex⁷. Moreover, parkinsonian patients⁸ and animals with basal ganglia lesions⁹ can compensate remarkably for movement disorders if they are provided with powerful moving visual stimuli to help them. It seems likely, therefore, that the basal ganglia are not responsible for visual or any other kind of sensory guidance of ongoing movements but instead select the most appropriate 'motor primitive'¹⁰ from a store of stereotyped basic motor programs¹¹. Thus, the basal ganglia may trigger motor actions, but are probably not involved in the moment-to-moment sensory guidance of their execution.

On-line control is probably a function of the cerebellum. In discussing the cerebellum, I shall first summarize the way in which it links hierarchical levels in the central nervous system (Fig. 2), then add more detail with respect to visual projections.

To guide voluntary-movement signals from the highest cortical level (on the scale first suggested by Hughlings Jackson¹²), prefrontal and posterior parietal association areas of the cerebral cortex (PPC, area 7) pass not directly to the motor cortex, Jackson's middle level, but via the pontine nuclei of the brainstem to the lateral cerebellar cortex (Fig. 2). From here they pass via the dentate nucleus to the nucleus ventralis lateralis of the thalamus, thence to the premotor cortex (PMC, area 6). Thus, the lateral cerebellum mediates between Jackson's highest and middle levels. Similarly the intermediate, paravermal cerebellum helps to transmit signals from the middle to the lowest levels. The intermediate cerebellar cortex receives inputs via the pontine nuclei from motor and premotor areas of the cerebral cortex and also from the spinal cord (spinocerebellar tracts) (Fig. 2). It projects via the interpositus deep nucleus of the cerebellum either back to motor cortex (with a relay in the ventro-lateral thalamus) or via the red nucleus to the spinal cord through the rubrospinal tract. Guidance and co-ordination of voluntary movements are therefore localized to the lateral and intermediate regions of the cerebellum. On the other hand, the vermis of the cerebellum, together with the flocculo-nodular lobe, is mainly concerned with the control of posture and reflex eye movements. It receives inputs from the vestibular system,

spinal cord and tectal visual and auditory pathways, and it projects via fastigial and Deiters nuclei to the vestibulo-spinal and medial reticulospinal postural control systems of the brainstem, and also via the medial longitudinal fasciculus to the brainstem motor nuclei supplying the external ocular muscles.

Posterior parietal cortex

Turning to the specific example of visual guidance of voluntary movements I will commence with a brief discussion of the inferior parietal lobule, area 7 of the posterior parietal cortex (PPC). Recently, experimental neuroscientists have come round to the view long held by clinicians that PPC is more than merely an association area for cutaneous and proprioceptive signals. It also receives auditory, visual, motivational and motor inputs¹³. In trained monkeys, Mountcastle and colleagues¹⁴ have shown that area 7 neurones discharge vigorously in response to their sensory inputs only if the animal subsequently directs its attention¹⁵, or an eye or limb movement, towards the stimulus¹⁴. The main effects of damaging the PPC are inattention to sensory stimuli and an inability to guide movements accurately towards them and it is probable that area 7 is an important site for the integration of passive retinal impressions with ocular-motor signals (corollary discharges) for the purpose of representing 'real' visual space, independent of eye movements¹⁶, as opposed to merely recording the retinotopic locus of targets where the position of objects changes with each eye movement.

It is a tribute to the intuition of Jackson that it has now become clear that the major output from the PPC is to the cerebellum and thence to his middle level, the motor cortex. In primates, the PPC provides the largest projection (apart from the motor cortex) to the pontine nuclei¹⁷, via the corticopontine tract.

Corticopontine tract

This is one of the largest pathways in the brain; in humans there are nearly 25 million corticopontine fibres on each side compared with only 1 million fibres in the corticospinal pyramidal tract. In lower mammals, such as the rat, almost the whole cerebral cortex projects to the pons¹⁸, but in primates only motor and premotor regions and the parietal cortex, particularly its posterior part (area 7, PPC) do so significantly¹⁷. Hence it is these cortical areas that are probably most involved in the visual guidance of movement. Glickstein and his colleagues have studied visual projections to the pons in both monkeys and cats.

The over-representation of the fovea which is found in most

cortical visual areas does not occur in the onward projection to the pons. In the occipital lobe high acuity for the centre of gaze is achieved by providing 10–100 × as many neurones to analyse the foveal representation as are given to the periphery; the representation of the centre is thus highly 'magnified'. But in the projection of visual information for the guidance of movement to the cerebellum it is probably more important to preserve accurate spatial relations between objects and their movements than details of their form. Hence the fovea is no longer accorded special privileges and its representation in the corticopontine pathway is 'demagnified'. Moreover, pontine neurones receiving visual projections are not especially sensitive to form (for example, the orientation of lines and edges) but are highly sensitive to movement, their most effective stimulus being multiple dots moving in a particular direction at a particular speed¹⁹. (They are unresponsive to stationary targets.)

In both the cat and the monkey visually sensitive cells in the pons which receive inputs from the parieto-occipital cortex, project, as 'mossy fibre' terminals, mainly to the contralateral cerebellar hemisphere²⁰, including the dorsal paraflocculus. Despite its name and proximity, the dorsal paraflocculus has little functionally to do with the flocculus or the vestibular system. The lateral cerebellum projects in turn, via the dentate nucleus and the ventrolateral nucleus of the thalamus, mainly to the premotor cortex.

Note that in neither rat, cat nor monkey is there evidence of a large projection of visual signals to the intermediate (paravermal and paramedian) cerebellar cortex. Significantly, these are the regions which receive the very rapidly conducting spinocerebellar tracts (Fig. 2); these provide moment-to-moment information about the lengths and tensions of muscles, the angles of joints (dorsal spinocerebellar tracts) and the state of excitability of spinal reflex pathways (ventral spinocerebellar tracts).

Intermediate cerebellum

Cajal first noted that all pyramidal tract neurones give collaterals as they pass through the pons, but there are also ten times as many fibres originating in motor cortical areas that project only to the pontine nuclei²¹. The destination of the mossy fibres originating in this part of the pons is the intermediate, paravermal, cerebellar cortex. So in addition to receiving spinocerebellar reports from the periphery, the intermediate cerebellum receives from motor areas in the frontal cortex (Fig. 2). These fibres may relay 'corollary discharges' representing the movements that the cortical motor centres are trying to achieve.

The early recognition that motor cortical and spinal feedback signals coincide in intermediate cerebellum²² gave rise to the idea that the main function of the cerebellum was to act as a comparator in a servo-control system that compares the desired position of a limb (input from motor cortex) with its actual position (input from spinal cord). The intermediate cerebellum projects to the rest of the brain via the interpositus nucleus. The lateral part of this nucleus feeds back to the motor cortex via the ventrolateral thalamus, and also projects more directly to spinal cord motor neurones via the red nucleus (Fig. 2).

Information processing

The neuroanatomy of visual pathways to the motor cortex provides only a basic framework for the question of how the cerebellum works. What essential transformations does the cerebellum carry out on visual signals to make them suitable for guiding movements? We have not yet progressed much further than the basic wiring diagram, but the introduction of techniques for working with trained animals has allowed the development of quantitative theories that try to take into account both the anatomical connections of the cerebellum and the detail we now possess about the electrical transactions between the cell types in the cerebellar cortex^{23,24}. The first task is to develop a theory of the cerebellum that is sufficiently realistic, yet detailed

enough, to be verifiable by experiment. But the mammalian motor control network is labyrinthine and there is no effective control theory for such complex systems.

The cerebellum as a comparator

Attempts to apply engineering techniques to the cerebellum have not so far been successful. As mentioned above, the coincidence of signals from motor cortex and spinal cord in the intermediate cerebellum suggests that the role of this region is to compare the desired position of a limb commanded by the motor cortex with its actual position signalled by the spinocerebellar system, and to issue necessary corrections to the motor cortex and spinal cord. Similarly, in the case of visuomotor control it was suggested that the lateral cerebellum compares the positions of target and hand, defined visually, and uses the 'error' signals so estimated to drive the hand towards its target²⁵. Hence it was hoped that the cerebellum would prove amenable to the techniques of linear systems analysis which have been so successful in the design of man-made control systems.

It seemed that the interactions of the many thousand parallel fibre inputs to each Purkinje cell, and the inhibition by each Purkinje cell of the nuclear cells underlying it (Fig. 1) could be a model of the comparator functions of the cerebellar cortex, but this idea now seems very naive. Those of us who tried to obtain direct evidence that the Purkinje cell acts as a comparator by recording from the cerebellum in monkeys trained to perform controlled movements were disappointed²⁶. Although the characteristics of Purkinje-cell discharges under these circumstances broadly support the comparator idea, they are also compatible with a host of other hypotheses about functions of the cerebellum. Unfortunately Purkinje cells do not label themselves unequivocally as comparators or indeed as performing any readily identifiable control operation at all.

Neurones in intermediate cerebellar cortex and the interpositus nucleus fire in close association with movements, at about the same time as those in the motor cortex, beginning about 30–40 ms before commencement of a movement²⁷. Their discharge matches the electromyogram (e.m.g.) of agonist muscles²⁸, and hence the velocity of each movement²⁷, fairly closely. These neurones respond to proprioceptive feedback, such as vibration of muscle tendons (a potent Ia muscle spindle stimulus) or jerks applied to the limb (torque pulses), as rapidly as 20 ms later²⁶. If a torque pulse perturbs normal tracking, the first line of defence is the spinal stretch reflex. This response (M1 in the agonist muscle e.m.g.) occurs ~20 ms after the jerk. It is followed by a second increase in e.m.g. activity at ~50 ms (M2). This is probably made up of two components: the 'long loop' stretch reflex uses a pathway via dorsal columns, motor cortex and pyramidal tract²⁹; and to this is added a longer latency response of the spinal cord, which is probably mediated by slower conducting group II muscle afferents³⁰. But these early responses have little power to compensate for the perturbation mechanically; the arm does not begin to correct until 100 ms later. By this time a cerebellar component has been added to the e.m.g. (M3)³¹. If the output of the intermediate cerebellum is temporarily inactivated by cooling the interpositus nucleus, mechanical compensation in response to a torque pulse is delayed and insufficient, hence the arm often begins to oscillate³². This disturbance probably results from the withdrawal of a potent cerebellar contribution to later responses in the motor cortex³³ and to the compensation effected by rubrospinal activity.

These findings imply that the intermediate cerebellum is concerned with the detailed execution of movements and helps to minimize disturbances that might throw them off course. They are therefore consistent with, but do not prove, the idea that paravermal Purkinje cells behave as comparators. The discharge of paravermal cells does not correlate well with the mismatch between commanded and actual position of the limb. Nonetheless, although the modulation of individual Purkinje cells may

be only loosely correlated with current errors in the execution of a movement, it is still possible that the overall response of the whole intermediate cerebellum is to act as a comparator for proprioceptive regulation of the execution of movements.

Lateral cerebellum as a predictor

The lateral cerebellum is more complicated. As mentioned above, many neurones there receive visual inputs and may discharge well before limb movements²⁶, so the responses of these cells are broadly consistent with the idea that they compare the position of a target with that of the limb. But here the difficulties that physiologists face when attempting to make use of engineering concepts in a precise way become apparent. In the case of the eye and limb, for example, it is not clear what co-ordinate systems such a comparator might use when attempting to relate the position of a target, defined visually, to that of the limb, reported by corollary discharge and proprioceptors³⁴.

The comparator idea cannot be applied too literally to the lateral cerebellum because on-line negative feedback is probably not the most important control strategy for the visual guidance of movement³⁵. The dorsal spino-cerebellar and magnocellular rubrospinal tracts which provide the main input from the spinal cord to the intermediate cerebellum are some of the most rapidly conducting pathways in the body, so the intermediate cerebellum can probably provide effective on-line servo-assistance helping to compensate for such disturbances as unexpected perturbations, changes of load and fatigue. In contrast, the visual system processes signals relatively slowly compared with the speed required of most movements and visual feedback is usually too slow to be used as the main control technique for guiding movements^{35,36}.

Instead, the cerebellum uses feed-forward control. Most movements are programmed before they start, and are then run off essentially independently of visual feedback except during their terminal stages. For stationary targets the required movement pulse is calculated from a comparison of the current positions of limb and target. So a comparator process is required, not as part of a feedback loop, but in the preparation of the control program for each movement. But if either target or observer are moving, successful preprogramming of movements demands accurate prediction of where target and observer will be at the end of the proposed movement, rather than time-consuming calculations of current visual error. Therefore the main signals that are likely to be found in the lateral cerebellum probably relate to this pre-programming process, and visual feedback may play a relatively minor part.

This is what has been found in practice. Lateral cerebellar neurones fire up to 400 ms before movements, sufficiently far in advance to be capable of contributing to preprogramming them³⁷. Many of these cells (particularly those in the dorsal paraflocculus) receive visual inputs²⁶ whose characteristics are consistent with an origin in the parietal cortex. The cells may be more sensitive to visual stimuli when the animal is intending to move, and their visual responsiveness may be switched off ('gated' out) during the early 'ballistic' phase of each preprogrammed movement during the period when visual signals should not be able to affect their progress. When a monkey is directing a movement towards a fixed target the discharge of some neurones in the lateral cerebellum correlates almost equally well with the initial separation of the limb and target (the visual error) as with the velocity of the subsequent movement²⁶. Under these circumstances the two signals are often very similar, although the first arises when the monkey decides to move, whereas the actual movement occurs 300 ms or so later. Hence it is not usually possible to tell unequivocally whether a lateral cerebellar neurone is responding to the visual information used for computing the movement, or is itself directing the action. This is partly a physiological problem which can be investigated experimentally, but partly it is merely semantic. In an area of the brain concerned with transforming sensory infor-

mation into motor control signals, there is no single point at which one can say that sensory input stops and motor output begins. It is more meaningful to attempt to elucidate the computations that occur in the transformation of one into the other.

When primates are following moving targets, positional visual error, that is, the separation of target and limb, stops being the most important control signal^{35,36}. Instead, subjects attempt to predict in various ways. If the target movement is regular and repetitive, subjects can match its movements almost perfectly, without any lag, after a short learning period. They appear to be able to lay down an internal model of its trajectory and play it back on demand³⁸; corrections are required only very occasionally. If the movements of the target are not predictable, subjects still attempt to make short-term predictions, but instead of tracking the moving target continuously they split the response into intermittent corrective movements. Under these circumstances the most important control signal for each movement is not the positional mismatch between target and limb that exists at the time (current error), because by the time this can be calculated it is likely to be out of date. Rather they use the velocity at which the target is currently moving³⁵, which sets the amplitude of each movement by allowing short-term predictions of where the target will be by the end of a proposed move.

The lateral cerebellum is probably crucial in both kinds of predictive processes. Holmes pointed out that lesions of lateral cerebellum particularly affect visually guided movements, leading to intention tremor and the tendency to hunt around the position of a target for which the patient is reaching. These symptoms do not imply that visual control is completely inactivated by cerebellar damage. If this were the case, the limb would never even approach its target. Instead, hunting implies that visual control is still operating, although slowly, but that the system becomes underdamped without adequate velocity control. The dramatic change produced by temporarily inactivating the lateral cerebellum in monkeys is just such a loss of velocity control³⁹. The movements of the animals become much larger and faster than normal and cease to be related to target velocity (see below). Moreover, when intact monkeys track sinusoidally moving targets the discharge of some neurones in the lateral cerebellum correlates with target velocity as well as it does with the velocity of each movement. This is exactly as expected if target velocity is the main influence on the motor output of these cells. Hence it is likely that the lateral cerebellum is important in generating short-term predictions, probably using target velocity as its main control signal.

Long-term predictions

The cerebellum is probably responsible for long-term predictions as well. If subjects attempt to track a target that is moving regularly in a predictable fashion they form memorized internal models of the required trajectory, which can then be produced on demand with little error or lag³⁶. Cerebellar lesions seem to prevent the acquisition of such stored skilled motor programs. In very well-trained monkeys some lateral cerebellar neurones can probably generate a motor output without any visual guidance: they discharge before movements made in anticipation of predictable target steps, in the absence of the sensory inputs which were needed to guide them during the learning period, and then become insensitive to their usual visual inputs⁴⁰. Furthermore the discharge of dentate neurones, unlike those in interpositus nucleus, modulates in relation to the intended direction of movements rather than to the movement which actually takes place, if the two are dissociated experimentally⁴¹. In these studies the direction of movement which the experimenter intended the monkey to make was taught over a period of weeks. The animal can therefore be said to have learnt an internal model of the required movement. In converse experiments, the response of a dentate neurone to identical movements was shown to reverse if the direction in which the animal intended to move was reversed⁴².

If one is expecting to make a movement against a certain force, then an appropriate internal model or 'motor set' is established to achieve it. If the conditions are unexpectedly changed, clearly one is unprepared and performs less well. A similar phenomenon can be observed in monkeys when the lateral cerebellum is cooled, when, despite lengthy training, the monkey is unable to generate the correct internal program appropriate for anticipating conditions with which it is very familiar⁴¹.

One way of summarizing the functions of the lateral cerebellum is that it is concerned with setting the goal of complete voluntary movements with regard to amplitude, direction and timing, particularly in relation to visual inputs. Thus, the lateral cerebellum is probably not involved in the actual execution of the movement (such as controlling the lengths of individual muscles), which is done by the intermediate cerebellum. To achieve successful control of whole movements, the lateral cerebellum probably contains, in addition to predictors, apparatus for generating the internal models that are required to program them on the basis of past sensory experiences. This makes it unnecessary for them to be closely guided during their execution. In this way new motor skills can be acquired.

Cerebellar plasticity

This brings us to the brink of the exciting topic of the role of the cerebellum in motor learning. It may seem surprising that I have so far discussed only the mossy-fibre inputs to the cerebellar cortex and have not mentioned the second main source of input, the inferior olive and its climbing fibres. This will especially upset those who believe that the climbing fibres, rather than the Purkinje cells, are comparators.

It seems fairly clear that individual climbing fibres cannot provide the moment-to-moment quantitative error signals that are required for controlling movement, because they fire only once or twice per second²³. More probably they play a part in mediating the long-term plastic changes in the cerebellum that underlie the generation of new motor programs, the establishment of an appropriate motor set and the acquisition of motor skills.

Each Purkinje cell receives inputs from between 250,000 and 1,000,000 mossy fibres via the parallel fibre axons of granule cells, but only a single climbing fibre, which makes 2,000–3,000 synapses with it (Fig. 1). It is probably necessary for only a small proportion of the parallel fibres impinging on a Purkinje cell to be active to elicit simple spike activity. It has been suggested that the function of the single climbing fibre input to each cell is to specify (by means of the complex spikes which

their activity produces) which small subset of the many parallel fibres that a Purkinje cell receives will preferentially excite it⁴⁴. If climbing fibres are eliminated early in development, parallel fibres fail to make proper contacts with Purkinje cells⁴⁵. It is possible, therefore, that continual production of complex spikes by climbing fibres is necessary to specify which parallel fibres will make effective functioning synapses with Purkinje cell dendrites. The mechanisms are not yet understood in any detail, but Masao Ito and colleagues have now provided good evidence that parallel fibre/Purkinje cell synapses are modifiable⁴⁶. Combining climbing- and parallel-fibre stimulation can alter the sensitivity of Purkinje cells to the input from the particular set of parallel fibres that were stimulated at the same time as the climbing fibre; but it leaves unchanged the excitability of synapses with the other parallel fibres that were not stimulated. These changes in excitability can last for more than an hour. Clearly they could mediate long-term modifications of cerebellar activity involved in motor learning.

Long-term adaptation of two reflexes has been studied in great detail from this point of view, the vestibulo-ocular and nictitating membrane reflexes. The vestibulo-ocular reflex is the mechanism that ensures that the gaze remains directed at the same point in space when the head is moved. After a few days wearing reversing spectacles, this reflex can be made to reverse⁴⁷, so that both head and eyes move in the same direction. This plastic change probably depends on the integrity of the flocculus of the cerebellum⁴⁸ and recording experiments in both primates⁴⁹ and rabbits⁴⁶ suggest that climbing fibre input to the Purkinje cells in this region is responsible.

Conditioned closure of the nictitating membrane (the 'third eyelid') in rabbits can be elicited by a flash of light after it has been paired sufficiently often with a mechanical or electrical stimulus applied to the cornea. This plastic change depends on the integrity of a small region of cerebellar cortex and is probably also brought about by climbing fibre discharges^{50,51}.

Clearly these mechanisms modifying the parameters of the vestibulo-ocular and nictitating membrane reflexes could also operate in less automatic control systems such as the visual guidance of movements. Gilbert and Thach have already shown that, when a monkey is learning a new movement, climbing fibre activity increases in parallel with the learning⁵². It is not yet known precisely what changes in Purkinje cell activity occur during the establishment of new motor sets, how new internal models for movements are generated, or how motor skills are acquired.

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Lead concentration changes in Antarctic ice during the Wisconsin/Holocene transition

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In order to assess current global lead atmospheric pollution, it is necessary to reconstruct the natural, pre-human atmospheric fluxes of this toxic heavy metal. The time variations of these fluxes over the past 27,000 years have been obtained from the analysis of an Antarctic ice core.

IN 1969, Murozumi *et al.*¹, measuring lead in Greenland recent snow and ancient ice, discovered that lead concentrations had increased from ~1 to ~200 pg Pb g⁻¹ during the past 3,000 yr. They also measured salts and silicate dust, and found a corresponding 100-fold increase in the Pb/silicate dust ratio during this same interval. On this basis they claimed that in earlier times most natural lead in the troposphere came from wind-entrained soil dust, while >99% of the lead now in the global troposphere of the Northern Hemisphere originates from human activities. Since this model was postulated, no other investigators have been able to control artefact lead contamination during sample collection and/or laboratory analysis sufficiently well to confirm or duplicate these Greenland data upon which the pollution model is based; however, the work of Murozumi *et al.*¹ initiated a revolution in polar snow and ice sample procurement and trace analysis procedures which resulted in a great improvement in the quality of subsequent measurements by other investigators of dust, salts and some trace metals other than Pb in polar recent snow and ancient ice^{2,3}.

During the following 12 years, other investigators were unable to measure correctly the low-concentration end (1 pg Pb g⁻¹) of the temporal lead curve in Arctic glacial ice, and published many erroneously high lead data ranging from ~50 to 2,000 pg Pb g⁻¹ for old Greenland ice⁴⁻⁶. They denied the existence of 100-fold man-made lead pollution effects in the Northern Hemisphere, and proposed instead that various large natural sources of lead, such as volcanoes, vegetation and rock volatilization, together with mechanisms which concentrate lead in sea spray or precipitation relative to air, could account for the enormous lead excesses present in today's north polar snows and Northern Hemisphere global atmosphere⁷⁻¹⁹.

The original characterization of the ancient (natural) end of the Greenland temporal lead curve by Murozumi *et al.*¹ did not start to become generally accepted until after it had been confirmed 12 years later by Ng and Patterson²⁰, using improved ultra-clean techniques. They also modified the dust model to include approximately equal contributions from volcanic fumes, on the basis of measurements that had been made of volcanic Pb/S ratios and global volcanic sulphur fluxes²¹. This Greenland temporal lead curve is the foundation which supports all knowledge concerning natural occurrences of lead in the Earth's atmosphere, oceans and biosphere, and the extent of industrial lead pollution of those systems.

The aim of the present study was to observe how lead concentrations changed in Antarctic ice when the Wisconsin/Holocene boundary was crossed, in order to test the global lead pollution model. The atmosphere became very dusty and salty during the final 10,000 years of the Wisconsin glacial stage, and then cleared during the following Holocene interglacial²²⁻²⁶. According to the global lead pollution model, changes in lead concentrations should follow changes in dust

concentrations and exhibit higher concentrations in late Wisconsin ice than in early and middle Holocene ice, there having been no industrial lead in the air to obscure the relationship. According to the natural-sources view, lead concentrations would be expected to exhibit no such dependence on dust concentrations in ancient polar ice, for contributions of lead to the ice from dust would be as minor then as they indisputably are now in the Northern Hemisphere.

The findings reported here show that changes in measured lead concentrations in Antarctic ice are congruent with the 30-fold changes in calculated lead concentrations expected from observed variations in dust concentrations across the Wisconsin/Holocene boundary. This makes it no longer possible to claim that even 2% of the lead now in the global troposphere of the Northern Hemisphere comes from natural sources.

Experimental techniques

Fourteen sections of the 905-m Antarctic Dome C ice core²⁷ have been analysed for lead. This core, which has been shown to cover the past 32,000 years²⁷, was taken at Dome C (77° 39' S, 124° 10' E; elevation 3,240 m; mean annual temperature -53.5 °C) during the 1977-78 Antarctic field season, using a thermal drill in a dry hole not filled with a wall-retaining fluid. The oxygen isotope profile of Lorius *et al.*²⁷ shows four main climatic stages: the Holocene stage from the surface down to ~380 m, the Wisconsin/Holocene transition from ~380 to 520 m, the last glacial maximum from ~520 to 680 m, and the earlier Wisconsin stage from ~680 m to the bottom of the core. Sections selected for lead analysis, packed in double, sealed polyethylene bags, were transported frozen from France to the California Institute of Technology (Caltech). Each section (10.5 cm in diameter; 20-30 cm in length) was cleaned by mechanically chiselling four to six successive veneers of ice in progression from the outside to the interior of each core section, inside a cooled, double-walled, nitrogen-flushed polyethylene tray placed inside the ultra-clean Caltech laboratory. The remaining inner core so obtained was typically 3 cm in diameter.

Each veneer layer and the inner core were melted overnight at room temperature in conventional polyethylene beakers. A non-acidified 10-ml aliquot was taken for subsequent analysis of various anions. Ultra-pure HNO₃ was then added to make a 0.1% HNO₃ solution, and the acidified solution was allowed to sit for 2 h. Aliquots of 50-100 ml were then taken for subsequent analyses of various cations and metals. These aliquots were refrozen until use. After being aliquoted as described above, the remaining 100-250 ml of each sample was then analysed for lead by thermal ionization isotope dilution mass spectrometry (IDMS), using ultra-clean laboratory methods.

Both the chiselling and lead IDMS procedures were similar to those previously described²⁸. Much lower lead blanks were achieved in this study because of improvements in the pro-

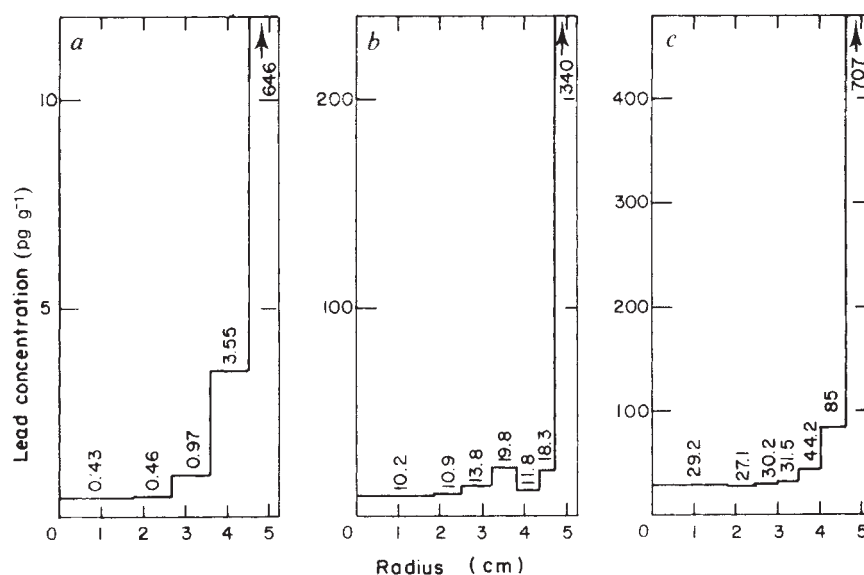


Fig. 1 Measured lead concentration as a function of radius in three sections of the 905-m-deep ice core obtained by thermal drilling in a non-fluid-filled hole at Dome C, East Antarctica. *a*, 451.74–452.01 m; *b*, 545.00–545.26 m; *c*, 670.38–670.57 m.

cedures: overall lead contamination introduced by chiselling and chemical treatment ranged from 30 to 60 pg, compared with 140–400 pg in the earlier study. When compared to the total lead content of the inner cores, the overall lead contamination correction ranged from <10% for Wisconsin ice cores containing 5–30 pg Pb g⁻¹, to 75% for Holocene ice cores containing 0.3–1 pg Pb g⁻¹.

The quoted precision of our data is based on an overall evaluation of relative contributions by contamination errors and is thought to be ~10% for high Wisconsin lead concentrations and ~50% for the very low Holocene lead concentrations.

Character of the data

Analyses were made of all veneers taken from 10 of the 14 core sections (some of the veneer layers from four other core sections were not analysed). Figure 1 shows the variations of lead concentration as a function of radius for three of the completely analysed core sections. For all core sections except the 775.58–775.82 m section, measured concentrations in the outermost veneer were found to be in the range 340–2,300 pg Pb g⁻¹ (for the 775.58–775.82 m section a much higher value, 45,000 pg Pb g⁻¹, was observed). The lead concentrations in the outermost layers are thus much lower than those observed by Ng and Patterson²⁰ for two sections of the Byrd ice core (20,000–30,000 pg Pb g⁻¹), which, like the Dome C core, was thermally drilled in a dry hole. We observed a sharp drop to low lead concentrations in second veneer layers (3–85 pg Pb g⁻¹), followed in most core sections by a plateau for subsequent inner veneers (Fig. 1). This shows that in these cores, interfering amounts of surface contamination added in the field during drilling and packaging were not transferred to the central portions of the core sections. However, for three core sections (Table 1), Pb concentrations decreased slightly between the last veneer layer and the inner core. For these three cores, the inner-core values must be regarded as upper limits.

Lead data from old Antarctic ice have previously been reported for two Holocene Byrd ice core sections, 1,490 and 2,010 yr old²⁰, and for a block of coastal Wisconsin blue ice whose age was estimated to be 10,000 yr (ref. 28). The reported concentrations (<1.4, <2.2 and <1.75 pg Pb g⁻¹, respectively) are consistent with our Dome C data in Table 1.

Aluminium, non-sea-salt sulphate and sodium concentrations have not yet been determined in the core sections analysed here, but values for these constituents measured in other core sections^{23,26} provide us with interpolated values for these constituents, which are used to evaluate the relative contributions of soil dust, volcano fumes and sea salts to the total lead concentrations measured in the Dome C core (Table 1).

Dome C lead profile

Figure 2 shows that lead concentrations were rather low (~1 pg Pb g⁻¹) 27,000 yr ago, and then rose to very high values (up to 30 pg Pb g⁻¹) during the late Wisconsin (last glacial maximum). They remained high for ~12,000 yr (from ~26,000 to 14,000 yr BP), and then declined rapidly to very low values (~0.5 pg Pb g⁻¹) within <2,000 yr (from ~14,000 to 13,000 yr, that is, during the Wisconsin/Holocene transition). They then remained very low (0.3–0.9 pg Pb g⁻¹) during the Holocene.

Lead from dust

Interpolated aluminium concentrations in the ice (Table 1) were ~30 times higher during the late Wisconsin than during the Holocene (~70 ng Al g⁻¹, peaking at 150 ng Al g⁻¹ in the late Wisconsin, compared with ~2.5 ng Al g⁻¹ in the Holocene).

Aluminium concentrations provide an index of the natural lead concentrations associated with it in humus and soil minerals. Pb/Al ratios in different igneous rock types at the

Table 1 Measured lead concentrations and estimated aluminium, sodium and sulphate concentrations in the Antarctic Dome C ice core

Depth (m)	Estimated age* (yr BP)	Inner-core Pb concentration (pg g ⁻¹)	Al† (ng g ⁻¹)	Na† (ng g ⁻¹)	SO ₄ † (ng g ⁻¹)
172.75–172.93	3,846	0.76‡	2.7	19	75
300.42–300.72	7,587	0.47	2.6	17	98
373.42–374.10	9,785	0.94‡	2.2	13.5	88
451.74–452.01	12,160	0.43	6.9	44	114
476.17–476.34	13,020	0.32§	5.2	43	113
500.39–500.60	13,900	3.81	12.6	70	126
527.06–527.31	14,950	10.5§	43	86	167
545.00–545.26	15,705	10.2	76	116	180
602.09–602.34	18,145	11.4	62	99	149
658.07–658.27	20,590	14.0§	70	112	204
670.38–670.57	21,120	29.3	41	90	170
704.06–704.27	22,640	15.2§	43	107	167
775.58–775.82	25,860	7.2‡	19	82	131
796.81–797.09	26,830	1.25	8.0	76	128

* J. P. Benoist, personal communication. This chronology is the best available for the Dome C core.

† Interpolated from Al, Na and SO₄ concentrations given in ref. 26 for 50 sections of the Dome C core. Al concentrations were determined by instrumental neutron activation, Na and SO₄ by ion chromatography.

‡ For this section, no definite plateau was obtained; the inner core value must therefore be considered to be an upper limit.

§ For this core section, only the inner core has been analysed.

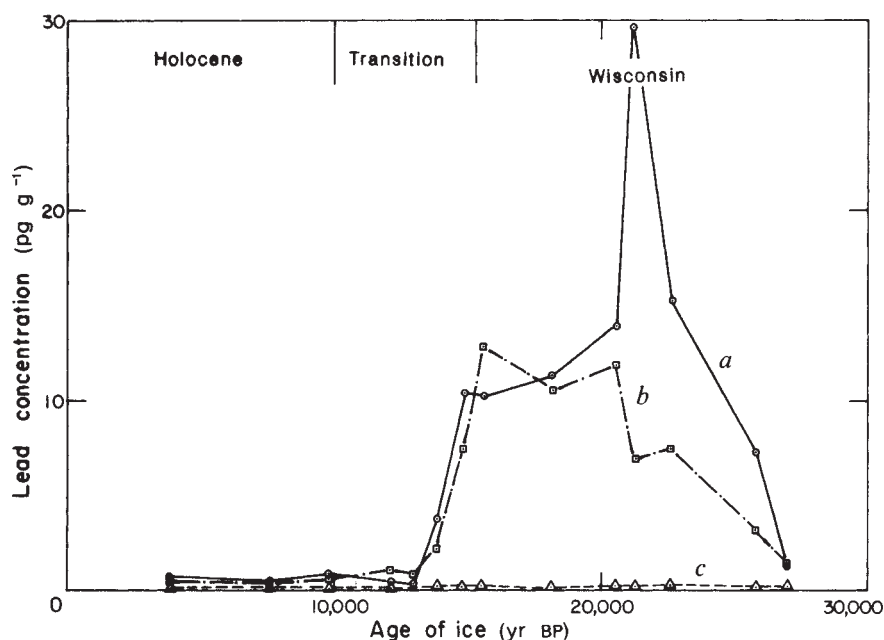


Fig. 2 Variations of lead concentration across the Wisconsin/Holocene boundary in the Dome C ice core. *a*, Measured lead concentrations in the centre of the analysed core sections; *b*, estimated lead contribution from dust, calculated from interpolated aluminium concentrations in the ice; *c*, estimated lead contribution from volcanoes, calculated from interpolated non-sea-salt sulphate concentrations in the ice. The estimated lead contribution from sea salts, calculated from the interpolated sodium concentration in the ice, is in the range 10^{-4} – 10^{-3} pg Pb g^{-1} , and thus cannot be shown in this plot.

Earth's surface do not vary by more than a factor of three from the mean composition, and the same is true for soils developed from those different rock types, compared with global mean soil compositions²⁹. Natural Pb/Al ratios determined in dust in marine atmospheres at various locations differ by less than a factor of 2 from mean values for either the Earth's crust or global soil²⁹. Isotopic studies of lead in soil horizons show that most of the industrial lead emitted to the atmosphere of the Northern Hemisphere and then returned to remote non-agricultural and non-urban soil during the past century has accumulated in humus within the uppermost 3 cm, where it accounts for approximately one-third of all lead in that soil layer³⁰. The estimated flux of natural soil dust lead through the global atmosphere is $\sim 1,400$ tonne yr^{-1} (ref. 29).

Figure 2 shows that natural soil lead values (curve *b*), estimated from aluminium values in the ice ($\text{Pb/Al} = 1.7 \times 10^{-4}$)²⁹, faithfully track lead concentrations measured in ice for both the Wisconsin and the Holocene, despite the fact that the aluminium values are not actual concentrations in the ice sections analysed for lead, but interpolated values. It must be emphasized that for the late Wisconsin, the use of interpolated values could result in errors of up to a factor of three in the estimation of lead from dust, as aluminium values exhibit rather large variations during the last glacial maximum^{23,26}. This is the most likely explanation of the fact that the highest lead value in the ice (30 pg Pb g^{-1} for the 670.38–670.57 m section) does not correspond to the highest soil-dust lead value in Fig. 2.

Soil-dust aerosols entered the ice as a result of having served as giant condensation nuclei for the formation of snowflakes^{31,32}. Similar mechanisms act on volcanic sulphate and sea-salt aerosols. It is highly improbable that large concentrations of natural non-particle lead gases would have been present in the late Wisconsin atmosphere, but were not transferred to the snow, because such gases are unstable and are easily transformed into particle residues. Gaseous lead emitted from volcanoes has only a brief atmospheric existence before it is sequestered by elemental sulphur aerosols²¹. Organic compounds such as lead alkyls rapidly decompose in the atmosphere to ionized trialkyl molecules, which are sequestered by existing aerosols³³. If such major sources of natural aerosol lead had been present in significant concentrations, they would also have been incorporated into snow, and the close correspondence between dust lead and observed total lead shown in Fig. 2 would have been obscured. It was not, and this constitutes irrefutable proof that

most of the natural lead in the global atmosphere originated from soil dust, during both the late Wisconsin and the Holocene.

Lead from volcanoes

Volcanic lead is not emitted to the atmosphere in the form of lead-rich silicate particles, but is instead attached to sulphur aerosols which subsequently become oxidized. The global concentration of volcanic sulphate in marine air ($\sim 250 \text{ ng m}^{-3}$) can be derived by two different methods²⁹: (1) a steady-state concentration in marine air of $\sim 400 \text{ ng volcanic SO}_4 \text{ m}^{-3}$ can be calculated from the volcanic sulphur emission flux (1.7×10^7 tonne yr^{-1}) in a sulphur emission budget model²¹ and the assignment of a 2-week residence time in the troposphere; and (2) direct measurements of non-sea-salt sulphate concentrations in marine air ($0.8 \mu\text{g SO}_4 \text{ m}^{-3}$)^{34,35} can be multiplied by the fraction of this sulphate estimated to be volcanic ($\sim 13\%$)³⁶ to yield a concentration of $\sim 100 \text{ ng volcanic SO}_4 \text{ m}^{-3}$. This suggests that the proportion of non-sea-salt sulphate, excluding industrial SO_2 , assigned a volcanic origin (13%) is valid within a factor of ~ 2 . The volcanic Pb/S ratio can be multiplied by this value of 13% to provide an index of natural volcanic lead associated with non-sea-salt sulphate concentrations in ice. The Pb/S ratio has been measured in fumes from six volcanoes, leading to an effective value of 7.4×10^{-5} (by weight)²¹. This ratio, multiplied by the output of volcanic SO_2 to the global atmosphere, yields $\sim 1,200$ $\text{tonne volcanic Pb per yr}$.

Levels of non-sea-salt SO_4 in Dome C ice²⁶ were only slightly greater during the late Wisconsin than in the Holocene (~ 1.5), so not much change in lead concentration would be expected if a major share were contributed by volcanoes. The reported mean non-sea-salt SO_4 /sea salt SO_4 ratio of ~ 1.6 in Dome C Holocene ice is ~ 10 times greater than the ratio of these constituents measured in marine air^{34,35}. This results from a rather strong relative decrease in sea-salt aerosols compared to sulphate aerosols in going from marine to interior regions such as the Dome C location. For example, non-sea-salt SO_4 /sea-salt SO_4 in recent surface snow increases from 0.3 to 2 on going 430 km inland from the coast^{26,37,38}. When the above volcanic Pb/S ratio and per cent volcanic SO_4 are used to index volcanic lead associated with non-sea-salt sulphate in Dome C ice, the amount of such volcanic lead calculated to be present is $\sim 0.5 \text{ pg volcanic Pb g}^{-1}$ for the late Wisconsin and $\sim 0.3 \text{ pg volcanic Pb g}^{-1}$ for the Holocene. For the late Wisconsin, this is $\sim 1\%$ of the measured total lead, and for the Holocene about half of the

measured total lead (compare curves *c* and *a* in Fig. 2). This lack of correlation of volcanic Pb with changes in total Pb in the ice shows that in general, excepting occasional brief effects from extremely large eruptions, volcanic contributions of natural lead to the Earth's atmosphere during the late Wisconsin were minor compared to those from soil dust. During the Holocene it appears that about half of natural lead came from soil dust and half from volcanoes. The latter proportions agree with present global fluxes of volcanic lead (1,200 tonne yr⁻¹) and soil-dust lead (1,400 tonne yr⁻¹) to the troposphere.

Lead in sea salts from ocean spray

Sea salts do not appear to constitute a major source of natural lead to the atmosphere. Sea-salt concentrations were about five times greater in late Wisconsin ice (250 ng NaCl per g ice) than in Holocene ice (50 ng NaCl per g ice)²⁶. In order to index the concentrations in ice of natural lead provided by these salts, lead concentrations occurring in surface sea waters thousands of years ago (~0.5 pg Pb per g sea water)³⁹ must be increased to account for the effects of enrichment during the formation of sea spray. Isotopic studies of the natural and industrial origins of lead in salts, soil dust and anthropogenic aerosols in marine air, dry deposition and rain, and of lead in sea water^{40,41} have shown that lead-rich aerosols, introduced to the sea by rain, are sequestered by oils at the sea surface, where they are in part re-introduced to the atmosphere in sea spray before the lead dissolves out of the particles into the underlying sea water. The proportion of these lead-rich particles concentrated by surface tension into sea spray is ~5,000 times greater than the proportion they would otherwise occupy in well-stirred sea water, so that sea-spray salts in today's mid-ocean atmospheres account, for example, for 8% and 20% of total lead in the North and South Pacific Easterlies, respectively²⁹. However, lead isotope tracers show that most of this lead is industrial and is attached to anthropogenic carbonaceous particles⁴⁰. As the only form of natural aerosol lead not immediately soluble in sea water is that in soil humus and minerals, the sea-salt index for contributions to ancient global atmospheres is reduced ~50-fold from the present value after industrial lead-rich particles are eliminated.

Sea-salt-derived lead concentrations calculated as described above from the interpolated sodium concentrations given in Table 1 are ~4×10⁻⁴ pg Pb g⁻¹ for late Wisconsin ice and

~1×10⁻⁴ pg Pb g⁻¹ for Holocene ice. This is ~10⁻⁵ of the measured total lead for the Wisconsin, and ~10⁻³ for the Holocene. These extremely small contributions show that sea salts are not a significant source of natural lead to the global atmosphere.

Conclusions

We have obtained the first description of the variations in the lead concentration of Antarctic ice from 27,000 to 4,000 yr BP. This has allowed us to delineate the natural, pre-human atmospheric fluxes of this highly toxic element. Natural lead concentrations have varied greatly in Antarctic ice during the time period studied: lead levels were high (up to 30 pg Pb g⁻¹) during the late Wisconsin (last glacial maximum), and very low (~0.5 pg Pb g⁻¹) during the Holocene.

These time variations faithfully track variations in soil-dust lead calculated from aluminium concentrations in the ice, thus proving that most of the natural lead in the global atmosphere originated from soil dust, during both the late Wisconsin and the Holocene. The lead contribution from volcanoes, calculated from sulphate concentrations in the ice, was negligible during the late Wisconsin, but accounts for about half of the measured total lead during the Holocene. The lead contribution from the oceans is insignificant throughout the period studied.

These data confirm that before humans began significantly to affect the atmosphere, there was no excess of lead above that contributed by soil dust and volcanoes. Our results establish that >99% of the lead now in the troposphere of the Northern Hemisphere originates from human activities.

Similar reliable studies will need to be done for other toxic metals and metalloids, such as Cd, Cu, Zn, Ag, As and Se, in order to establish their past natural background fluxes. Only when these fluxes are accurately known will it be possible to assess the magnitude of the current global pollution of these elements in both hemispheres.

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Structure of the receptor for platelet-derived growth factor helps define a family of closely related growth factor receptors

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The primary structure of the receptor for platelet-derived growth factor (PDGF), determined by means of cloning a cDNA that encodes the murine pre-PDGF receptor, is closely related to that of the v-kit oncogene product and the receptor for macrophage colony stimulating factor (CSF-1). Common structural features include the presence of long sequences that interrupt the tyrosine-specific protein kinase domains of each molecule. The PDGF and CSF-1 receptors also share a characteristic distribution of extracellular cysteine residues. Ubiquitin is covalently bound to the purified PDGF receptor, the human gene for which is on chromosome 5.

SEVERAL growth-stimulating peptides and their respective receptors have now been identified and characterized. Although the mechanism by which these molecules regulate proliferation is unknown, some insight into their function has come from the discovery that they are structurally and functionally similar to several of the retroviral oncogene products. Through these comparisons specific functional domains of several of the growth factor receptors can be identified. For example the receptors for insulin^{1,2}, macrophage colony-stimulating factor (CSF-1)³, and epidermal growth factor⁴ have, in their amino acid sequences, recognizable domains for tyrosine-specific protein kinase, an activity that is associated with both proliferation and transformation of cells⁵.

One of the known growth factors, platelet-derived growth factor (PDGF), specifically stimulates the proliferation of mesenchymal cells. PDGF is similar to insulin, insulin-like growth factor I, and epidermal growth factor in stimulating tyrosine kinase activity⁶⁻⁹, but it is distinctive in its ability to act early in the transition from the quiescent state to G₁ and to facilitate the subsequent actions of other growth factors¹⁰. It also stimulates a number of growth associated responses such as cytoskeletal rearrangement¹¹, turnover of phosphatidylinositol¹², and enhanced expression of a family of genes including the *c-myc* and *c-fos* proto-oncogenes^{13,14}. The first step in activating the cellular responses associated with PDGF-induced mitogenesis is the interaction of PDGF with its 180 kDa cell surface receptor¹⁵⁻¹⁸. The receptor is also required for the transformation of cells by the simian sarcoma virus oncogene, *v-sis*, which encodes one of the two polypeptide chains of PDGF¹⁹. To gain insight into the mechanism by which the PDGF receptor mediates the responses to PDGF, we purified the receptor, determined amino acid sequences of portions of the molecule, and deduced the complete amino acid sequence of the receptor from a full length cDNA clone. In addition we determined its chromosomal position in the human genome.

Receptor purification and sequencing

The PDGF receptor was purified from BALB/c 3T3 cells in its activated form which is phosphorylated on tyrosine residues⁶⁻⁹.

The purification was based on sequential affinity chromatography steps using immobilized wheat-germ agglutinin and antiphosphotyrosine antibodies (see Fig. 1 legend), procedures that were modified from those used previously to purify analytical quantities of PDGF receptor²⁰. In PDGF-stimulated cells the predominant tyrosine-phosphorylated protein that is recognized by antiphosphotyrosine antibodies is the PDGF receptor^{9,20}. Thus the progress of the purification could be followed by Western blot analysis using polyclonal antiphosphotyrosine antiserum (Fig. 1a). By this approach the receptor protein was purified to near homogeneity as assessed by Coomassie stain (Fig. 1b) or by silver stain (not shown). An estimate of the extent of the purification was 4000-fold based on the intensities of the antiphosphotyrosine antibody Western blot signals. By this procedure approximately 400 µg of receptor was purified from 2,000 roller bottles of BALB/c 3T3 cells with an overall yield of 20%. Prior to sequence determination, the eluate from the antiphosphotyrosine antibody column was applied to a preparative SDS polyacrylamide gel and the receptor was eluted from the 180,000 (180K) M_r region.

Two independent receptor preparations (40 and 130 picomoles per preparation) were used for determination of the N-terminal amino-acid sequence of the receptor. In both cases there were two distinct phenyl-thio-hydantoin (PTH)-conjugated amino acids at each cycle of Edman degradation and these were present in approximately equimolar amounts. One of these sequences was identical to the amino terminal sequence of ubiquitin²² and the other sequence (Table 1) represented the amino terminus of the PDGF receptor (see below). To determine internal sequences of the receptor, four independent trypsin digestions of the protein were performed on three separate preparations. A total of 29 peaks isolated by HPLC were analysed by amino-acid sequencing on a gas phase sequencer. Of these, 15 peaks contained homogeneous peptide species. The sequences of these peptides are presented in Table 1. One of these peptides, peptide 2, was isolated independently from each of the four tryptic digests and another, peptide 3, was isolated independently from two of the digests. Peptide 7b was found to overlap with peptide 7a, possibly the result of an abnormal tryptic cleavage after a glutamine residue. Thus the experimentally determined sequences were derived from ten independent internal peptides and from the amino terminus of the receptor.

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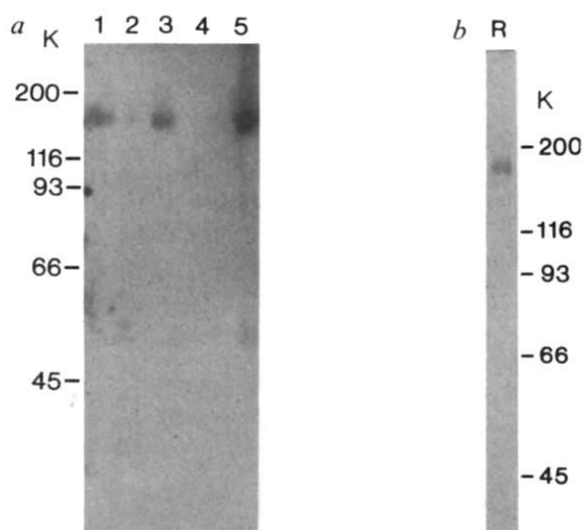


Fig. 1 *a*, Antiphosphotyrosine antibody Western blot analysis of fractions from purification steps. Aliquots from the following fractions of the purification procedure were analyzed by antiphosphotyrosine antibody Western blot: lane 1, 100 μ g of cell lysate protein; lane 2, 100 μ g of flow through fraction of wheat germ agglutinin sepharose chromatography; lane 3, 1 μ g of protein eluted from wheat germ agglutinin sepharose; lane 4, 1 μ g of flow through fraction of antiphosphotyrosine-sepharose chromatography; lane 5, 200 ng of receptor protein specifically eluted from antiphosphotyrosine sepharose. *b*, Coomassie stain of purified PDGF receptor (lane R). An aliquot (200 ng) of the eluted receptor fraction run in lane 5 of Fig. 1*a* was analysed by Coomassie stain of a 7.5% polyacrylamide gel.

Methods. BALB/c 3T3 cells grown in roller bottles were incubated with 5 nM PDGF for 3 h at 4 °C and were then solubilized in cold Tris-buffered Triton X-100 (20 ml per bottle) containing 100 μ M sodium metavanadate, 50 mM sodium chloride, 5 mM EDTA, 10 mM Tris-HCl (pH 7.4), 1% Triton X-100, 1 mM phenylmethylsulphonyl fluoride and 1 mg ml⁻¹ bovine serum albumin. The lysates were centrifuged at 39,000 g for 1 h at 4 °C and supernatants were added slowly to a 30 ml column of wheat-germ agglutinin coupled to Sepharose CL-4B. After washing with 10 column volumes of the Tris-buffered Triton solution the column was eluted with 120 ml of 0.3 M *N*-acetyl glucosamine in Tris-buffered Triton. The eluates were diluted to the original volume and added to a 5 ml column of immobilized antiphosphotyrosine antibody^{9,20} at a flow rate of 30 ml h⁻¹ at 4 °C. The column was sequentially washed with 10 column volumes of Tris-buffered Triton without albumin, and with Tris-buffered octyl- β -D-glucoside (20 mM octyl- β -D-glucoside substitute for Triton). Phosphotyrosine-containing proteins were specifically eluted with a buffer containing 40 mM phenylphosphate, 3.3 mM Tris-HCl (pH 7.4), 30 mM NaCl and 20 mM octyl- β -D-glucoside. Fractions from each step were applied to 7.5% SDS-polyacrylamide gels, and transferred to a nitrocellulose filter which was sequentially incubated with antiphosphotyrosine antibody and radiolabelled goat anti-rabbit antiserum to detect the phosphotyrosine-containing proteins²¹. For amino acid sequence analysis, the purified fractions from the anti-phosphotyrosine sepharose column were concentrated, reduced, alkylated by iodoacetamide treatment⁵⁸ in the presence of lithium dodecyl sulphate and were further purified by preparative SDS gel electrophoresis followed by electroelution⁵⁷. The overall yield of purified receptor was approximately 20% (0.5–1.0 μ g of receptor per roller bottle).

PDGF receptor cDNA sequence

A pool of 128 39-base oligonucleotides (Fig. 2 legend) was synthesized based on the sequence of peptide 2, and used to screen oligo(dT)-primed cDNA libraries prepared from mouse placenta and cultured NR6 mouse fibroblasts²³. Thirty-six strongly hybridizing clones were selected: 17 from the mouse

Table 1 Experimentally determined peptide sequences of the PDGF receptor

Peptide	Sequence	Preparation	Recovery (pmol)
(1)	XVD(I)PLH(V)PYDHQ (E) (F)	IIb	10
(2)	(L)VEPVTIDYLFQVPS	I, IIa, IIb, III	15, 10, 27, 10
(3)	LLETGLDVE(I)AEL(H) (P) (P)	IIb, III	13, 12
(4)	(Y)VSELILV	III	8
(5)	YEI(R)	III	12
(6)	DQLVLG	I	30
(7a)	TLG(S)GAFGQVVEATA (E)	III	7
(7b)	(V)VEATA(H)GL (Y)	I	21
(8)	XXPIIITIEYXFYGDV(D)Y (G)	I	14
(9)	XXDFGLA(R)	I	30
(10)	SDHPAIL(R)	III	12
(11)	LV(I)TPP(G)P(E)FV(L)XISSTF (N-term) (G)	A, B	50, 12

Experimentally determined peptide sequences of the PDGF receptor. Purified PDGF receptor was electroeluted from a preparative SDS polyacrylamide gel (see Fig. 1 legend), precipitated⁵⁶, and digested with trypsin. The digestion mixtures were fractionated on a reverse phase C18 column (120 T-TSK, LKB) developed with a gradient of acetonitrile in 0.1% trifluoroacetic acid. Peptide samples were sequenced by automated Edman degradation on a gas phase sequencer (Applied Biosystems) using the 02N VAC program and methanolic/HCl conversion chemistry. The PTH residues were identified and quantitated using a cyano HPLC column procedure similar to that described by Hunkapillar⁵⁷. Total net yields were calculated at each cycle after correction for background. The recoveries based on determination of PTH-amino acids are shown. The repetitive yields were calculated by linear regression analysis through all quantitated cycles except those containing serine, threonine, histidine, and cysteine. The repetitive yields were 93–97%. The peptide sequences shown were derived from three different receptor preparations and four independent digestions. The starting amounts of PDGF receptor prior to digestion were: preparation I, 130 pmol; IIa, 200 pmol; IIb, 65 pmol; and III, 50 pmol. N-terminal sequence analysis was performed on the intact receptor (preparation A, 130 pmol, preparation B, 40 pmol). Residues are indicated by single letter amino acid codes. Residues identified but not quantitated are enclosed by parentheses. X indicates that no residue could be identified. The position of each numbered peptide in the predicted cDNA sequence is indicated by the numbers within the small squares in Fig. 2.

placenta library (denoted λ -MP) and 19 from the NR6 library (λ -N). To establish that we had isolated PDGF receptor cDNA sequences, two 800 bp clones (λ -N14 and λ -MP6) were sequenced using the dideoxy nucleotide chain termination procedure^{24–26}. The open reading frames of the two clones overlapped for 354 nucleotides and included a sequence that coded for the peptide used to design the oligonucleotide probe. These clones hybridized to the other 34 clones. The largest clone, λ -N16, contained a 5.2 kilobase (kb) insert which was potentially large enough to encode the PDGF receptor. To confirm the identity of the clone, two additional oligonucleotide probes were synthesized based on the sequences of tryptic peptides 7a and 8 (see Fig. 2 legend). As expected, both probes strongly hybridized to the λ -N16 clone.

The complete nucleotide sequence of the cloned cDNA insert in the λ -N16 clone extends for 5,110 nucleotides, and is flanked by a poly(A) sequence at the 3' end (Fig. 2). An open reading frame of 3,327 residues (nucleotides 106–3,432) is flanked by 105 nucleotides of 5'-untranslated sequence and 1678 nucleotides of 3' untranslated sequence. A polyadenylation signal (ATTAAA) 17 nucleotides upstream from the poly(A) sequence is found at the 3' end of the cDNA. The first ATG codon, found in this open reading frame at position 139, matches the consensus sequence for a translation initiation site²⁷. A 17-amino-acid sequence that is homologous to the experimentally determined amino-terminal sequence of the purified PDGF-receptor protein (Table 1) begins 32 amino acids downstream from the initiation codon. Seventeen of the eighteen experimentally determined amino-terminal residues matched the sequence predicted from the cDNA (Fig. 2). Thus the leucine residue 32 amino acids from the first ATG codon is the amino terminal residue of the

Fig. 2 Nucleotide sequence and deduced amino-acid sequence of mouse pre-PDGF receptor cDNA. Oligonucleotide probes based on peptides 2, 7a, and 8 (Table I) of the PDGF receptor were synthesized. The DNA sequences were designed on the basis of published codon usage frequencies^{59,60}. Full-length oligonucleotides (peptide 2) or overlapping partial oligonucleotides (peptides 7a and 8) from coding and noncoding strands were chemically synthesized⁶¹. The probe sequences for peptide 2 included all possible codon combinations for amino acids with two alternative codons. Other codons for this probe and for probes corresponding to peptides 7a and 8 were predicted on the basis of codon usage statistical data. The screening of cDNA libraries and selection of clones is described in the text. The complete sequence of the λ -N16 clone is shown in this figure. Nucleotides are numbered at the left. Amino acids are numbered over the line starting at Leu1 of the receptor sequence, and preceded by a 31 amino acid signal peptide. Peptide sequences derived from purified receptor preparations are underlined and numbered according to Table 1. Potential sites of N-linked glycosylation are overlined. The putative single transmembrane region is demarcated by a black bar. The ATTA AAA box close to the polyadenylated 3' end of the cDNA is underlined.

Methods. Synthetic probes were radio-labelled by 5'-end phosphorylation⁶³ using T4 polynucleotide kinase (PL Biochemicals) and [γ -³²P]ATP (Amersham >5,000 Ci mmol⁻¹). Synthetic probes corresponding to peptides 7a and 8 were further labelled by filling in the complementary sequence using *Escherichia coli* DNA polymerase I (Klenow fragment, Boehringer-Mannheim) and [α -³²P]dCTP and [α -³²P]dATP (>5,000 Ci mmol⁻¹, Amersham). The resulting radiolabelled oligonucleotides were separated from radionucleotides by gel filtration through Sepharose G-50 (Pharmacia). This procedure yielded specific activities of $3\text{--}6 \times 10^8$ c.p.m. per μg . Total poly(A)-containing RNA from cultured mouse NR6 cells and mouse placenta was prepared by the guanidine thiocyanate-LiCl method⁶⁴ followed by passages through an oligo(dT)-cellulose column⁶⁵. A clone library was constructed as described⁶⁶ using the λ gt10 vector system (Vector) and was screened by hybridization to the radiolabelled oligonucleotide corresponding to tryptic peptide 2. Hybridization to the λ -phage containing nitrocellulose filters was carried out at 40 °C for 16 h under low stringency conditions; 20% formamide, $5 \times \text{SSC}$ ($1 \times \text{SSC} = 150 \text{ mM NaCl}$, $15 \text{ mM Na}_3\text{ citrate}$). Nucleotide sequence analysis was carried out by subcloning of restriction fragments of λ -N16 recombinant phage clone into M13-based cloning vectors followed by primed DNA synthesis on single-stranded DNA templates in the presence of dideoxynucleotide triphosphates^{24,26}.

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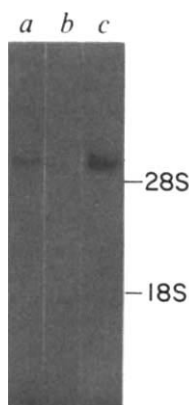


Fig. 3 Identification of PDGF receptor mRNA by Northern blot analysis. Total poly(A)-containing RNA was isolated from cultured human skin fibroblasts (a), A431 cells (b), and NR6 mouse fibroblasts (c) using the guanidine-monothiocyanate-LiCl method of cell homogenization⁶⁴ followed by oligo(dT)cellulose chromatography⁶⁵. The RNA from NR6 cells was re-chromatographed on oligo(dT) cellulose. RNA (4 µg) was heated for 10 min at 65 °C in the presence of formamide and formaldehyde and then subjected to electrophoresis on a 1.2% agarose gel which contained formaldehyde⁶³. After transfer to nitrocellulose the samples were hybridized at 42 °C with nick-translated⁶⁶ λ-N16 cDNA insert (5,134 base pairs, Fig. 2) in 50% formamide, 5×SSC and 50 µg ml⁻¹ denatured salmon sperm DNA. The filters were then washed at 50 °C with 0.2×SSC, 0.1% SFS. Exposure was for 5 days at -60 °C using an intensifying screen. Calf thymus ribosomal RNA was used as a size standard. (4,718 bp and 1,874 bp).

mature PDGF receptor. The amino acid residues between the ATG codon and the amino terminus of the mature protein are hydrophobic and likely represent the signal peptide sequence necessary for transport of the nascent pre-PDGF receptor precursor into the lumen of the endoplasmic reticulum²⁸⁻³⁰.

The experimentally determined amino acid sequences of the ten tryptic peptides could be located in the predicted amino acid sequence of the open reading frame of the cDNA insert of clone λ-N16. The peptide sequences matched the cDNA-derived amino acid sequence for all 120 experimentally determined amino acids except for residue 12 of peptide 8 which was identified to be phenylalanine but predicted from the cDNA to be arginine. The cDNA sequence also predicted the tryptic cleavage sites at the amino termini of the peptides. Taken together these findings show that the cloned cDNA insert in the λ-N16 clone represents the coding sequence of the PDGF receptor mRNA.

In addition to the signal sequence, the deduced 1098 amino acid polypeptide sequence contains features characteristic of cell surface glycoprotein receptors. These include a stretch of 25 hydrophobic amino acids characteristic of a membrane-spanning domain³¹ (underlined by a black bar in Fig. 2), which is flanked at its carboxyl side by a basic sequence typical for the junction between the membrane and cytoplasmic domains of cell surface receptors^{1-4,32-33}. Fifteen consensus sequences for asparagine-linked glycosylation (Asn-X-Ser/Thr) are distributed with a preference for the amino-terminal and presumably extracellular half of the sequence (11 potential sites). Nineteen cysteine residues are found distributed over the pre-PDGF-receptor sequence (boxed type in Fig. 2).

Single receptor mRNA

To determine the size of the PDGF receptor mRNA, Northern blot hybridization experiments were carried out using the cDNA insert of λ-N16 clone as hybridization probe. Figure 3 shows that a single band is visualized in cytoplasmic poly(A)⁺ RNA of NR6 mouse fibroblasts, in contrast to multiple mRNA species

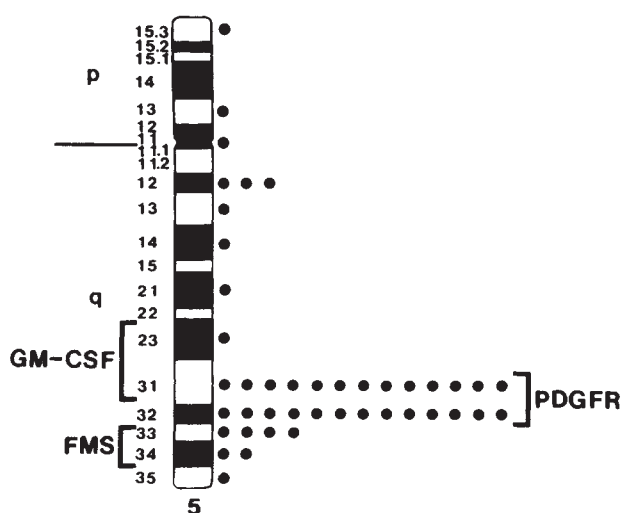


Fig. 4 Autoradiographic silver grain distribution along chromosome 5 after *in situ* hybridization with λ-N16 cDNA insert (ideogram from ISCN 1985)⁶⁷. The methods have been described⁶⁸. Brackets on the left mark the published localizations of the gene for GM-CSF^{35,36} and *fms*^{36,37}. Specific labelling at bands 5q31→5q32 indicates position of PDGF receptor locus.

reported for other receptor tyrosine kinases such as EGF and insulin receptors^{1,2,4}. This single mRNA species is approximately 5.3 kb in size, indicating that our cloned cDNA represents most if not all the messenger RNA encoding the mouse pre-PDGF receptor.

In a survey of mouse tissues and cell lines (data not shown) relatively high levels of the 5.3 kb mRNA species were found in kidney and placenta, and lower levels were detected in brain and testes. Likewise, all 3T3 mouse fibroblasts tested contained a similar band. However, 3T3-L1 adipocytes contained only 20% as much receptor message as 3T3-L1 fibroblasts, and NR6 cells contained only 30-40% as much as BALB/c 3T3 fibroblasts. High levels of PDGF receptor mRNA were also found in PDGF-responsive human foreskin fibroblasts, but not in human epidermal carcinoma A431 cells which are known to be devoid of PDGF receptors³⁴ (Fig. 3). Human term placenta and a 16-day mouse placenta contained elevated levels (2-4-fold) of PDGF-receptor mRNA when compared with mRNAs from earlier stage of pregnancy (20th-week human placenta and 10th-day mouse placenta).

Chromosomal location of PDGF receptor

The chromosomal location of the human PDGF receptor gene was determined by *in situ* hybridization of the 5.2-kb mouse PDGF receptor cDNA fragment (λ-N16) to chromosome preparations. Of 120 cells analysed, 26 (22%) had silver grains on bands (5q31→5q32) accounted for 10% (26/259) of total grains scored. No other chromosomal site was labelled above background. This location of the PDGF receptor gene is between the loci of the granulocyte-macrophage colony-stimulating factor (GM-CSF) gene (5q23→5q31)^{35,36} and the *c-fms* gene (5q33→5q34)^{36,37}.

Southern blot analysis of DNA prepared from ten human×Chinese hamster somatic cell hybrids confirmed the localization of the PDGF receptor gene to chromosome 5. The hybrids carrying human chromosome 5 all contained the 7.3-kb human-specific *Hind*III fragment and a 2.6-kb human fragment visible after longer exposure (not shown). Three fragments, 17 kb, 9.3 kb and 2.2 kb, were present in Chinese hamster DNA and in all hybrids. Chromosome 5 was the only human chromosome that showed perfect concordance with human PDGF receptor sequences. Every other human chromosome could be ruled out by at least two discordant hybrids.

a

PDGF-R	-31	MGLPGVIALVLRGQLLLSVL--MLLPQTSRGLVITPPGPEFVNLSTFVLTSGSAP
c-fms/CSF-1-R	1	MG-PGV---LLL---VATAWH-G-QGI---PVIEPSVPELVKPGATVTLR VNGS
consensus		MG-PGV---L---L---V---W-G-Q---VI-P---PE-V---T---L---G---
PDGF-R	28	VMEQMSQVPMQEAAMNDGTFSSVLTNTVGGDTGEYVYNNSLGPELSEKRIYIF
c-fms/CSF-1-R	48	VENHGPPS-PHNTLYS--DGS--SSILSTNNATFQNTGYRTEPGD--P-LGSSAAIHL
consensus		V-W-----P-----DG--SS-L---N-T---TG-Y---P-L---I---
PDGF-R	88	VPDPTMGLPMQSEDLFIFYDTDTTITPRTVDPLETLHEKKVDI-PL--HVPYDHO
c-fms/CSF-1-R	101	VKDPARPWNVLAEVVFVEQDQAL--LP--LLTDPVLEAGVSLVRGRPLRHNTNYSFS
consensus		V-OP-----E-----D-----P---TDP-LE-----V---PL--H-Y---
PDGF-R	145	--RGFTG---TFEDKT-YI--XTTIGOREVDSOTYVYVSLQVSSINVSNAVQTV-VRQ-G
c-fms/CSF-1-R	158	PMHGFTIHRKFIQSQDYQ--SALMGGKRVMSISRLKQVQVPGPPALTLVPAELVRIG
consensus		--GFT---F---Y---G-R-V-S---V---V---VR-G---
PDGF-R	197	ESITIRIVMGNDVWVFWYTPRMKSGRLVEPVT-DYLFVGPVSRIGSLIHPAELSOSG
c-fms/CSF-1-R	218	EAAQIVYSSASVD--VNFDFVLFQHNNT-KLAIPQSDFHNNRYQKVL-LNLQDVFQHAG
consensus		E---I---D---VNF-----L---P-D-----L---L---G---
PDGF-R	256	TYTIVSVSVNDHDEKAINISVIENGYRRLLETLDGVIEAELHRSRLRV-VFAYMP
c-fms/CSF-1-R	275	NYS--VASNVQKGHSTSMFRVYV-EAYLNLSSQNLQEVTVGEGMLKVMV-EAYPL
consensus		-Y--V---V---V---V-E-Y-L---D---P---L---V-V---AYP---
PDGF-R	315	SVL--MLKDMRTLGDSGAGELVLRNMSSTRYVSELLVVRVYSEAGYTYMRAFHEDDEV
c-fms/CSF-1-R	332	QGFNNTYLGPFSDDHPEKLANATTKDTY-RHTFTLSLRLKPSSEAGYRSLANPQGW
consensus		--W-----L---T---R---L-L-R-K-SEAG-Y-A---
PDGF-R	374	QLSKLVNVPVRVLESESHPANGQITIRGRGMPQPNVTSTRLK--PRKISPT
c-fms/CSF-1-R	391	ALTFELTRYPPPEVSVITFINGSG--TLLAASGYPPQNVTLQSGHTDRDEAQLQ
consensus		-L-F-L---P---V---G---T---G---PQPNTV---R---
PDGF-R	433	PLGNSKEE--SQLENTVTFWEEDQYEVVSTLRLHVDPLSVRLQNSMG--GDSQEV-
c-fms/CSF-1-R	449	WMDPYPVPLSQE--PFHKVTV-QSLTVEITLE--HN-QTYE--RAHNSVSGS-SWAFI
consensus		---E-SQ-E---Q---V-TL---H-Q---NS-G-G-S---
PDGF-R	490	--TVVPHSLPFK
c-fms/CSF-1-R	500	PISAGAHTHPPDEFLFTP
consensus		-----P---

b

PDGF-R	514	VLSIIIIIMWKKRIPRIRIRKXVIESVSSDGHETVYVDPVGLPPLSTWELPRDQIVLGRIT
c-fms/CSF-1-R	528	LIILLLLLLYKIKQPKYQVRWKIIEYIGNSYFETIDPTGLPYGEKWEFPRNNLQFQKIT
v-kit	1	EEITGMNLT--DPTGLPYGEKWEFPRNNLQFQKIT
c-src	213	YITSRTQFNSLQQLVAIYKSHADGLCHRLTTCPTSKPQTGLAKDAWEIPRESLRLEFYK
PDGF-R	574	SGAFGQGVVEATAFGLGKQHVIRKVAVKMLK--STANADEKALMSSEKIMSHLGQHWMT
c-fms/CSF-1-R	588	LGAAGAFGKVVVEATAFGLGKQHVIRKVAVKMLK--STANADEKALMSSEKIMSHLGQHWMT
v-kit	33	LGAAGAFGKVVVEATAFGLGKQHVIRKVAVKMLK--STANADEKALMSSEKIMSHLGQHWMT
c-src	273	LGGGFGFGLVMMGNTN--G--TTRVAFKTLKPGNMS--E-AFLGLAQMVK--KLRHEK
PDGF-R	633	VNLLGACTKGGPIYITETCYRGGDLVDFVHMKHFTLDRHSKMKAFDEPVG
c-fms/CSF-1-R	647	VNLLGACTKGGPIYITETCYRGGDLVDFVHMKHFTLDRHSKMKAFDEPVG
v-kit	92	VNLLGACTKGGPIYITETCYRGGDLVDFVHMKHFTLDRHSKMKAFDEPVG
c-src	322	LVQLVIAVVEEPIYITETCYRGGDLVDFVHMKHFTLDRHSKMKAFDEPVG
PDGF-R	693	SLPSHLNLTGESDGGYMDMMSKDESIDVPLDMQGLIYADTESPYMAPYDNYVPSAP
c-fms/CSF-1-R	707	KYVRARUSGSSGGVDTYVFNRPVST--NDSFSEGLKDEGRPL
v-kit	150	SSCN--DSTN--E--YNDNMKPGVS--TVVPT--KAD--KRRSARIGSYI--E
c-src		
PDGF-R	753	TYRATLINQSPY--LSYTDLVGFSSQVANGMDFLASKNCYHRDLAARNVLI--CEGKLVKIC
c-fms/CSF-1-R	751	-----ELRDLTHFSQVANGMDFLASKNCYHRDLAARNVLI--CEGKLVKIC
v-kit	188	QDVTVAIMEQDELADLELSTQVAKGMFLASKNCYHRDLAARNVLI--CEGKLVKIC
c-src	359	-----R--PQLDMARQDIA--SMAFYERMTYVLRDLAARNVLI--CEGKLVKIC
PDGF-R	812	DFGLARDIRDSNYISKGSYICPLKWMAPESIFMCTYVTVSDVWSGILLWEFTLGTPT
c-fms/CSF-1-R	796	DFGLARDIRDSNYISKGSYICPLKWMAPESIFMCTYVTVSDVWSGILLWEFTLGTPT
v-kit	248	DFGLARDIRDSNYISKGSYICPLKWMAPESIFMCTYVTVSDVWSGILLWEFTLGTPT
c-src	404	DFGLARDIRDSNYISKGSYICPLKWMAPESIFMCTYVTVSDVWSGILLWEFTLGTPT
PDGF-R	872	YPELPMNDQFYNAIKKGYRMAQPARHASDEITYEIMOKCWEKFEITRPPPSOLVLELRLG
c-fms/CSF-1-R	856	YPELPMNDQFYNAIKKGYRMAQPARHASDEITYEIMOKCWEKFEITRPPPSOLVLELRLG
v-kit	308	YPGMDVDSKYEIKKGYRMAQPARHASDEITYEIMOKCWEKFEITRPPPSOLVLELRLG
c-src	463	YPGMDVDSKYEIKKGYRMAQPARHASDEITYEIMOKCWEKFEITRPPPSOLVLELRLG
PDGF-R	932	GYKKKYQVDEEFLRSDHPAILKSGARFPGHSLRSLDTSVSLVYTAVDNENSDNYII
c-fms/CSF-1-R	916	EDKRRDYVTLNPLSSRSGSGSSSELEEESSSEHLLTCEQGQDIAOPLLPNNTYFC
v-kit	368	KNE
c-src	522	STE PQYQPGENL
PDGF-R	992	PLPDPKPDVADEGLPEGSPSLASSTLNEVNTSSTISCDSPLELQEEPQQAEPQAELQDPQ
PDGF-R	1052	USGCGPLAAEAEDSFL

Fig. 5 a, Comparison of the amino acid sequence of the extracellular domain of the pre-PDGF receptor with the sequence of *c-fms*/CSF-1 receptor³. The predicted extracellular amino-terminal halves of both proteins are aligned and gaps are introduced for optimal alignment. The putative signal sequences are included in the comparison. Clusters of conserved amino acids between the proteins are boxed and all cysteine residues are shaded. Amino acid numbers are given preceding each line of sequence. Leu1 of PDGF receptor is the experimentally determined amino-terminus of the protein. As the precise location of the amino-terminus of the *c-fms* protein is still unknown, the numbering of *c-fms* starts at the initiation methionine³. **b**, Comparison between the cytoplasmic domain of PDGF receptor and other tyrosine kinases. The predicted amino acid sequence of PDGF receptor is compared with that of the closely related genes, *c-fms*/CSF-1 receptor³ and *v-kit*³⁹. The sequence of the *c-src* gene⁶⁹ is also included for comparison. Only the cytoplasmic sequences of PDGF receptor and *c-fms*/CSF-1 receptor and portions of the respective transmembrane domains (underlined) are aligned. Residues which are identical in PDGF receptor and other genes are boxed. Gaps, shown by hyphens, were introduced for optimal alignment. Asterisks demarcate residues thought to be involved in nucleotide binding. The open arrow indicates the conserved tyrosine acceptor site homologous to Tyr416 of pp60^{v-src}⁶⁹.

Discussion

The 180 K protein which we have identified as the PDGF receptor has tyrosine kinase enzyme activity²⁰ and is the same size and has a similar isoelectric point as the PDGF receptor identified by ¹²⁵I-PDGF cross-linking experiments^{9,18}. Its overall structure, deduced from the cDNA sequence, is consistent with that of a receptor and includes a transmembrane domain, a tyrosine kinase domain, and potential sites for N-linked glycosylation in the extracellular domain. In previous studies we showed that high affinity ¹²⁵I-PDGF binding copurifies (10,000-fold) with this protein²⁰. The protein and its message are present in cells known to respond to PDGF and both are absent from cells that are unresponsive (Fig. 3). The yield of PTH-amino acids determined in the sequence analyses of the amino terminus and the internal peptides, suggests that the sequences are, in fact, from this protein and not from a minor contaminant (see Table 1).

The protein product predicted by the cDNA clone would have an apparent *M_r* of 120 K after removal of the signal sequence. In recent experiments we have shown that glycosidase treatment of biosynthetically labelled PDGF receptor removes sialic acid and N-linked oligosaccharide moieties and causes a 30–40 K decrement in apparent molecular weight (T.D. *et al.*, in preparation). Other post-translational modifications such as

addition of O-linked oligosaccharide, ubiquitin, or phosphate groups may contribute to the remainder of the difference between the apparent weight and the predicted weight.

Inspection of the predicted primary structure of the PDGF receptor reveals structural features common to other cell-surface receptors for growth factors (Fig. 5). These include a single stretch of predominantly hydrophobic amino acids (residues 500–524) capable of serving as a transmembrane anchor sequence, an amino-terminal extracellular domain (residues 1–499) that should contain the ligand binding region (Fig. 5a) and an intracellular domain (residues 525–1,067) carrying the enzymatic phosphotransferase activity (Fig. 5b).

The PDGF receptor tyrosine kinase domain, which can be identified by homologies with the transforming proteins of the *src* family of tyrosine kinases^{1–5}, is divided into two distinct regions (residues 572–662 and 767–919) separated by a 104-amino-acid sequence (residues 663–766) that is unrelated to known tyrosine kinase sequences (Fig. 5b). Inserted sequences that interrupt kinase domains at analogous locations of the *v-fms*³⁸, *c-fms*³ and *v-kit*³⁹ genes have been recently identified. Notably the insertion of *v-kit* shows significant homology with the insertion of PDGF receptor (24 identities out of the 79 residues of the insertion of *v-kit*) as compared with only six identities between the somewhat shorter insertion of *c-fms*/CSF-1 receptor and PDGF-receptor. All three insertion sequences

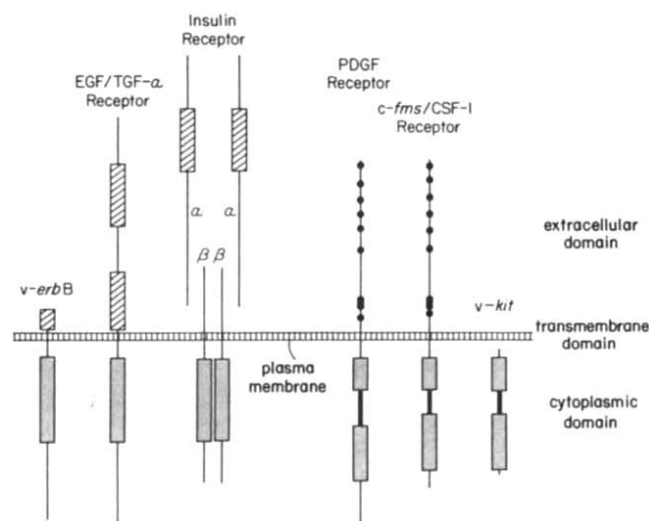


Fig. 6 Topological comparison of the two groups of tyrosine-kinase receptors and their oncogenic variants. All proteins shown are oriented so that their carboxy-termini are in the cytoplasm. Hatched boxes indicate regions that are rich in cysteine residues. Protein domains that share high homology with other tyrosine-kinases of the *src* gene family are shown by stippled boxes. Solid lines show stretches of sequences inserted within the tyrosine-kinase regions of PDGF receptor, *c-fms*, and *v-kit*. The distribution of cysteine residues in the extracellular domains of PDGF-receptor and *c-fms*/CSF-1 receptor is indicated by closed circles, whereas hatched boxes show cysteine-rich repeat domains found in the extracellular ligand-binding regions of EGF receptor, insulin receptor, and HER2/*neu*.

are relatively hydrophilic. Considering its position, the inserted sequence may play a role in cytoplasmic substrate binding or in modulation of kinase activity.

The expected tyrosine kinase landmarks are found in the split PDGF receptor kinase domains. The amino-terminal kinase domain (residues 572–662) contains residues which are thought to be involved in formation of the nucleotide binding site including Lys₆₀₂ and a Gly-X-Gly-X-X-Gly sequence⁴⁰. The sequence of this region (residues 572–662) is highly homologous with members of the *src* family of kinases, the closest match being *c-fms* (68% homology) which is thought to be the receptor for CSF-1⁴¹. The second kinase region (residue 767–919) contains most of the conserved kinase sequences, including the homologous sequences surrounding the major site of autophosphorylation (Tyr 416) of pp60^{v-src}⁴².

Other portions of the PDGF receptor sequence, in addition to the kinase domains, show striking homology to the *c-fms*/CSF-1-receptor sequence. The extracellular domains have a very similar pattern of cysteine distribution (Fig. 5a) and have a 30% sequence homology which is due mainly to short conserved sequences and conservation of all of the cysteine residues. Significant homology (34%) is also found in the stretch of 47 amino acids (residues 525–571) between the transmembrane region and the kinase domain (Fig. 5b). The two kinase domains of the PDGF receptor are the most homologous to *c-fms*/CSF-1-receptor sequences (72% and 64% respectively). The sequence that interrupts the kinase domain (residues 663–766) and the 148-amino-acid carboxy-terminal region of the PDGF receptor are the least homologous to the corresponding *c-fms*/CSF-1-receptor regions (8% and 13% respectively) (Fig. 5a). Taken together these findings show that the PDGF receptor and the *c-fms*/CSF-1-receptor are closely related, both in general structural features (cysteine distribution, location of kinase domains and presence of insert regions) and in specific sequences. The intracellular domain of the PDGF receptor is also closely related to the sequence of the *v-kit* transforming gene of HZ4 feline retrovirus³⁹ (Fig. 5b). This homology is apparent in both the

tyrosine kinase sequences (63% homology) and in the short region of *v-kit* which is between the *gag* sequences of the viral fusion protein and the amino terminus of the kinase domain (53% homology). Thus the normal cellular homologue of the *v-kit* oncogene may encode a receptor that is closely related to the receptor for PDGF and CSF-1.

Based on the structural features of the PDGF receptor and on the presented sequence homologies (Fig. 5) it is possible to divide the known receptor tyrosine kinases into two subgroups which are schematically depicted in Fig. 6. The first subgroup, which includes the PDGF receptor, *c-fms*/CSF-1-receptor³, and the *v-kit* protein³⁹ (and its putative normal cellular homologue), is characterized by kinase domains with long inserted sequences, and in the case of the PDGF receptor and *c-fms*/CSF-1-receptor, a similar pattern of cysteine distribution in the extracellular domain. The other subgroup includes the EGF receptor⁴, the insulin receptor^{1,2} as well as HER2/*neu* oncogene^{43–45}, which is thought to be a receptor of an as yet unidentified ligand. Their common structural elements include partially homologous extracellular domains with characteristic cysteine-rich regions and uninterrupted intracellular kinase domains (Fig. 6). It is of interest that unlike insulin and EGF, both PDGF⁴⁶ and CSF-1⁴⁷ are disulphide-linked dimeric molecules. Whether the structural similarity of these ligands is related to the structural similarity of the receptors remains to be determined.

A striking finding in the experimentally determined PDGF receptor sequence was the presence of two amino terminal sequences. One of these was the predicted amino terminus of the processed protein encoded by the cDNA clone, and the other was the sequence of the 8.5 K peptide, ubiquitin. The most likely explanation of these findings is that ubiquitin is covalently bound to the PDGF receptor, presumably through an amide bond between the carboxyl-terminal amino acid of ubiquitin and an ϵ -amino group of a lysine residue of the receptor. The role of this modification of the receptor is not known. Ubiquitination of other proteins appears to play a role in protein degradation⁴⁸ and specific ubiquitination of histones appears to be involved in transcriptional control^{49,50}. Recently Siegelman *et al.*⁵¹ have reported that ubiquitin is conjugated to the lymphocyte homing receptor, possibly in the extracellular domain. Thus there may be other surface molecules, possibly some receptors, that are modified by ubiquitination. It is possible that this post-translational modification plays a role in signal transduction or receptor processing.

The human PDGF receptor gene is located near the gene for the *c-fms*/CSF-1 receptor protein. Acquired partial deletions of this region of chromosome 5 are found in a number of haematologic disorders^{52–55}. Since the chromosomal breakpoints in these disorders cluster in bands 5q13→5q15 and 5q31→5q34^{52,55}, it is possible that in at least some of these patients there are alterations in the PDGF receptor gene. Whether these alterations are functionally important remains to be determined.

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LETTERS TO NATURE

Infrared point sources aligned with the SgrA* non-thermal radio source

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Recent observations^{1,2} have revealed point sources at wavelengths 0.7–1.0 μm and 1–5 μm , which are approximately aligned with the compact non-thermal radio source SgrA* in the galactic centre. The assembled 0.7–5 μm data indicate that the two point sources are at the same position and have spectral continuity; they are thus probably the same object. Here we discuss alternative interpretations of this infrared point source. If it is a foreground star, it must be a hot star surrounded by a circumstellar dust cloud. If it is an object at the galactic centre, an unorthodox extinction curve is required to derive an intrinsically hot black-body spectral distribution, $F_\nu \propto \nu^2$, over the 0.7–3.5 μm wavelength range. Under these circumstances the infrared emission may be the Rayleigh–Jeans tail of a hot star or star cluster, or thermal accretion disk with temperature $\approx 35 \times 10^3$ K.

The physical relationship between the infrared point sources^{1,2} and the SgrA* source^{3,4} is unclear, in that the former object or objects are possibly foreground sources which are not actually in the region of the galactic centre⁵. The observed flux levels from the results of Biretta *et al.*⁵ and Forrest *et al.*² over the frequency range $13.9 < \log \nu < 14.65$ (wavelength range $0.7 < \lambda < 3.75 \mu\text{m}$) are plotted in Fig. 1. We have used the conversion from AB (defined in ref. 6) to F_ν ($\text{erg cm}^{-2} \text{s}^{-1} \text{Hz}^{-1}$) given in ref. 6, $AB = -2.5 \log F_\nu - 48.60$ (a misprint in ref. 6 gives $+48.60$) for the $0.7 < \lambda < 1.0 \mu\text{m}$ data. In addition to the alignment in direction of the Biretta *et al.*⁵ object A (also called CCD2; refs 7, 8) and the object designated IRS16NW by Forrest *et al.*², we

note that there is spectral-flux continuity as well, before corrections for reddening are applied. The directional alignment within 0.2 arc s and spectral-flux continuity lead us to conclude that object A and IRS16NW are the same object. We adopt this conclusion, but we do not assume that this infrared source has any relationship to SgrA* (see also ref. 9).

The difficulty of interpreting the 0.7–5 μm observations of the infrared point source (object A + IRS 16NW) arises in attempting to correct the observed flux levels as a function of frequency for extinction and reddening due to intervening dust. There are two initial possibilities to be investigated: the infrared source (A + 16NW) may be a foreground star⁵, or it may lie in the vicinity of the galactic centre. We illustrate various possible intrinsic spectral-flux distributions of the infrared point source in Fig. 1, which result from applying a standard extinction law as a function of wavelength for assumed V-band extinctions ($\lambda = 0.55 \mu\text{m}$), $A_V = 10, 20$ and 30 mag . We have adopted the extinction curve of Becklin *et al.*¹⁰ plus van de Hulst no. 15 (ref. 11) in these corrections. The values of A_V represent the range of visual extinction that might be expected for a foreground star in the direction of the galactic centre, up to those values expected for sources in the galactic centre ($A_V \approx 30 \text{ mag}$; ref. 12).

The extinction corrections shown in Fig. 1 illustrate the difficulty of interpreting the infrared point source observations over the entire $0.7 < \lambda < 3.5 \mu\text{m}$ spectral interval. Spectral distributions resulting from the corrections for $A_V = 20$ and 30 mag are exceedingly steep between 0.7 and $1.0 \mu\text{m}$. We agree with Biretta *et al.*⁵ that there seems to be no natural physical explanation for such a steep spectrum at these wavelengths. It is this result that led Biretta *et al.*⁵ to suggest that their object A is a foreground star.

The recent results of Forrest *et al.*², however, complicate the interpretation of the infrared point source as a simple, early-type star. Our de-reddening calculations for $A_V \approx 10 \text{ mag}$ show that this star would have a large infrared excess at wavelengths beyond $\lambda \approx 1 \mu\text{m}$, presumably attributed to a circumstellar dust shell. This would be the natural interpretation of observations such as those shown in Fig. 1 (see ref. 13 and refs therein). We can determine, approximately, the nature of the hypothesized

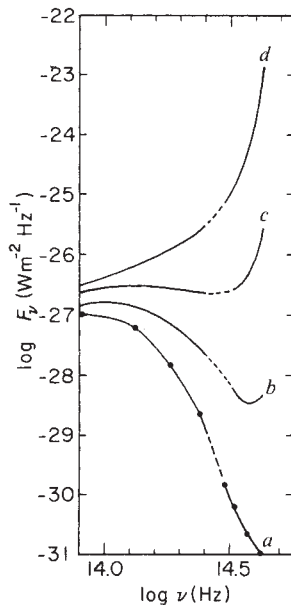


Fig. 1 Spectral flux distributions of the infrared point source. *a*, Observed flux versus frequency. *b*, *c*, *d*, Results for de-reddening by $A_V = 10, 20$ and 30 mag, respectively, using a standard extinction law as described in the text.

dust-enshrouded star from the observed fluxes. We assume that the $0.7\text{--}1.0\text{ }\mu\text{m}$ flux observations are due to the star, and that the longer-wavelength radiation originates from the circumstellar dust. Thus, the $\lambda \geq 1.2\text{ }\mu\text{m}$ data indicate the luminosity of the stellar object, as the dust absorbs the short-wavelength emission that cannot be directly observed and re-radiates that energy in the infrared.

The ratio of the Rayleigh-Jeans flux from the star (as measured at $\lambda = 0.7\text{ }\mu\text{m}$, $\nu_0 = 4.3 \times 10^{14}\text{ Hz}$) to the total integrated flux at $\lambda > 1\text{ }\mu\text{m}$ as an indication of luminosity, is

$$\frac{F_{\nu_0}}{F_T} = \frac{2\pi k T \nu_0^2 R^2}{c^2} \frac{1}{D^2} \frac{1}{4\pi\sigma T^4} \frac{D^2}{R^2} = \frac{2\pi k \nu_0^2}{c^2 \sigma T^3}$$

where T is temperature, R radius, D distance to the source, k is the Boltzmann constant, $1.38 \times 10^{-16}\text{ erg K}^{-1}$, and σ is the Stefan-Boltzmann constant, $5.7 \times 10^{-5}\text{ erg cm}^{-2}\text{ K}^{-4}\text{ s}^{-1}$. The accumulated observations indicate that the calculated parameters for the star would be $T \approx 2 \times 10^4\text{ K}$ (independent of distance), $R \approx 1.0\text{ }R_\odot$ and luminosity $L \approx 2 \times 10^2\text{ }L_\odot$ for an assumed distance of 5 kpc . A BOV star would have $T \approx 2 \times 10^4\text{ K}$, $R \approx 7.6\text{ }R_\odot$ and $L \approx 10^4\text{ }L_\odot$. Although no stars with the required combination of T , R and L exist, we do not rule out the existence of a hot star with a circumstellar dust cloud, as the estimate of total flux is a lower limit and the $0.7\text{--}3.5\text{ }\mu\text{m}$ observations may be consistent with such a model. In this interpretation, there is no natural connection with the SgrA* radio source. Is it merely by chance that such a peculiar, dust-enshrouded star lies almost precisely in the line of sight to SgrA*?

Having concluded that the data imply the existence of a hot black body in the direction of the galactic centre, an alternative interpretation results if we do not assume the standard wavelength dependence of extinction. We assume instead that all of the $0.7\text{--}3.5\text{ }\mu\text{m}$ data is to be attributed to emission from a hot black body, and that, therefore, $F_\nu \propto \nu^2$ is the intrinsic spectral distribution of the source over the observed infrared frequency range. Figure 2 shows the required extinction curve. This extinction curve would have $A_V/E(B-V) = 4\text{--}5$, if extrapolated to the V band, and would imply that the infrared point source has $A_V \approx 18$ mag. Extinction curves with unusually large total to selective extinction, and other supporting data, have been discussed elsewhere^{14–22}.

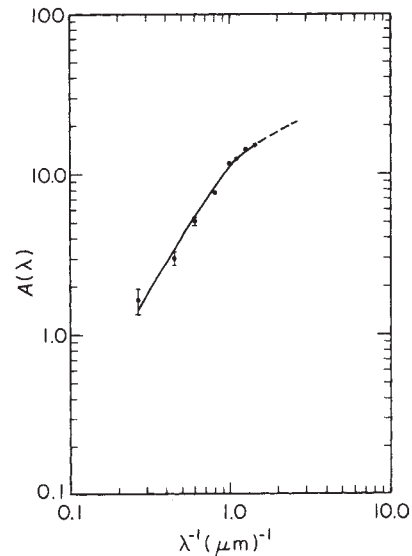


Fig. 2 The extinction law required to give a $F_\nu \propto \nu^2$ (Rayleigh-Jeans) intrinsic spectral distribution for the infrared point source.

If we adopt the extrapolation of the $3.75 < \lambda < 0.7\text{ }\mu\text{m}$ data to the ultraviolet with a $F_\nu \propto \nu^2$ black-body distribution, a luminosity of $L \approx 10^6\text{ }L_\odot$ results for $T = 3.5 \times 10^4\text{ K}$ (ref. 23). It is interesting that this luminosity is the same as that required to provide the infrared luminosity of the central 30 arc of the Galaxy¹⁸. However, the required photoionizing luminosity for the gas clouds in the central 3 pc of the Galaxy is $\geq 2 \times 10^7\text{ }L_\odot$ (ref. 23) and this value has to provide the total thermal radio emission from SgrA* (ref. 24), as well as the general infrared emission from this region¹⁸.

If SgrA* is the result of non-thermal activity of a massive compact object of mass $M \approx 10^6\text{ }M_\odot$ (ref. 25), or a more modest $10^2\text{ }M_\odot$ black hole^{9,26}, then perhaps the infrared point source is the Rayleigh-Jeans tail of a $T \approx 3.5 \times 10^4\text{ K}$ thermal accretion disk²⁷. Lacy *et al.*²⁵ explored detailed models for thermal accretion disks in an attempt to explain the total required photoionizing luminosity $L = 2 \times 10^7\text{ }L_\odot$, concluding that such a region would be brighter at $2.2\text{ }\mu\text{m}$ than any source observed. However, they could not rule out the existence of a source of luminosity with $L < 2 \times 10^6\text{ }L_\odot$. An alternative model might be a $T = 3.5 \times 10^4\text{ K}$, highly luminous star with $L \approx 10^6\text{ }L_\odot$ (refs 28, 29), although the coincidental association of such a luminous star with the other peculiar characteristics of the SgrA* source would seem unlikely.

None of the alternative explanations of the observations leads to conventional explanations of the infrared point source as either an object at the galactic centre or as a foreground star. We hope that this discussion stimulates the gathering of additional data.

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New determination of the angular diameter of Sirius

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We report a new determination of the angular size of α CMA (Sirius), which has yielded an equivalent uniform disk diameter of 5.63 ± 0.08 arc ms. This result represents the first measurement of a main-sequence star by amplitude interferometry and clearly demonstrates that accurate determinations of angular diameters are possible with a small-aperture (100 mm diameter) amplitude interferometer through the turbulent atmosphere. This observation is also the first independent check of any of the angular diameters measured with the Narrabri stellar intensity interferometer¹. Such confirmation is significant because the temperature scale for stars hotter than the Sun is based on the Narrabri data². The new result is in excellent agreement with the earlier value and was obtained in only ~2% of the observing time required for the intensity interferometer.

The observations were carried out using the Chatterton Astronomy Department's 11.4 m baseline prototype stellar interferometer³, which has been installed in the grounds of the Australian National Measurement Laboratory near Sydney. The recent completion of this instrument is a major step towards the goal of constructing a very-long-baseline interferometer having at least one hundred times the sensitivity of the Narrabri instrument. The prototype has a 150-mm siderostat mirror at each end of the fixed north–south baseline to direct starlight into a central laboratory through evacuated pipes. The central optical system reduces the wavefront tilts induced by atmospheric turbulence to ~0.1 arc s r.m.s. by means of 'active' mirrors, and it delays one light beam by an amount which compensates for the difference in arrival time of the starlight at the two siderostats before combining the two beams.

Interference is observed only when the two optical paths from the star to the beam-combining optical element are equal to within the coherence length of the starlight. For the measurements reported here the optical bandwidth was $\Delta\lambda = 0.3$ nm at a mean wavelength $\bar{\lambda} = 441.6$ nm. This corresponds to a coherence length ($\bar{\lambda}^2/\Delta\lambda$) of ~650 μ m. Following Tango and Twiss⁴, the interference is detected as a correlation signal C , which is equal to the square of the fringe visibility. In order to eliminate the effects of phase fluctuations, the correlation is determined by averaging the results of a large number of rapid samples made using photon counting techniques. As the path-equalizing retro-reflector is moved through the position of path equality the observed correlation passes through a maximum, and the resulting plot of correlation against position gives a 'delay curve'.

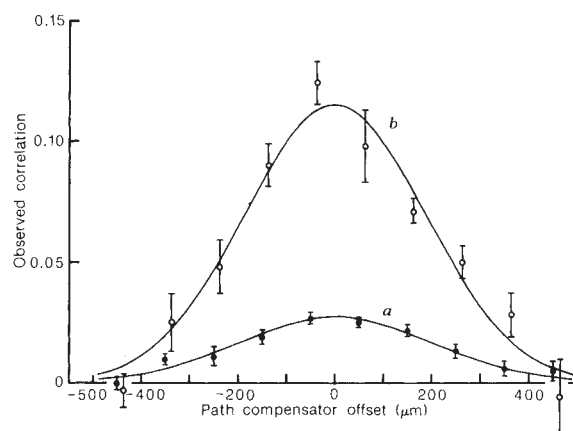


Fig. 1 Delay curves for α CMA (a) and β CMA (b). The ordinate is the observed correlation with no corrections applied. See text for further details.

Correlation was first observed with starlight on 1 October 1985, and as part of the programme to optimize the performance of the prototype, a series of delay curves has been measured for stars with different declinations and hour angles. The primary purpose was to determine the direction and length of the baseline vector from the positions of the maxima of these delay curves. The opportunity has also been taken to determine the angular size of α CMA, as the maximum of a delay curve gives the correlation at the equal-path position. At this stage the observed correlation is less than the true correlation, but, by normalizing it by the observed correlation from an unresolved star, the true value can be found.

The stars α and β CMA are close together in the sky (~5° apart) and their angular diameters were both measured with the Narrabri intensity interferometer¹. β CMA can be regarded as essentially unresolved, as far as the prototype interferometer is concerned. Its angular diameter was found to be 0.50 ± 0.03 arc ms at Narrabri, so the expected correlation for the prototype is 0.991 ± 0.001 and only a small correction need be applied.

We have measured delay curves for the two stars in close succession on six separate occasions. Figure 1 shows a pair of such curves measured on 20 February 1986. For all measurements we used an effective aperture diameter of 100 mm, a sampling time of 1 ms, and a 15-Hz bandwidth for the wavefront tilt correctors. The fitted curves in Fig. 1 are gaussian and the widths correspond to the coherence length set by the optical bandwidth. The baseline vector was not known precisely at the time of these observations and the curves shown in Fig. 1 have been shifted to place their maxima at the zero-offset position. This does not affect the peak values.

The ordinate in Fig. 1, the observed correlation, is the raw measurement with no corrections applied. It differs significantly from 0.991 for β CMA simply because the residual optical aberrations of the complex optical system have not yet been compensated with a figured surface, nor has any attempt been made to match the aperture to the prevailing value of r_0 (Fried's coherence diameter⁵). For each of the six pairs of delay curves we have taken the ratio of the maxima (α CMA/ β CMA) and corrected it for small calibrated losses due to irregularities in the motion of the path compensator (amounting to differential corrections of ~4%), and for the small difference from unity of the expected correlation for β CMA. The corrected ratio corresponds to the true correlation C for α CMA, and allows the angular diameter θ of the equivalent uniform disc to be determined from $C = [2J_1(x)/x]^2$, where $x = \pi d\theta/\bar{\lambda}$ and d is the length of the projected baseline.

The results of these observations are summarized in Table 1 which also lists the Narrabri results for comparison. The agreement between the two sets of results is remarkable, and suggests

Table 1 Angular diameter of α CMa

Date	Equivalent Uniform-disk angular Diameter (arc ms)	Wavelength (nm)	Observing time (h)
Narrabri, 1966–70 ¹	5.65 ± 0.17	438.5	12.1
	5.84 ± 0.23	443.0	6.4
	5.57 ± 0.09	443.0	11.0
	5.54 ± 0.17	443.0	9.4
	Weighted mean = 5.60 ± 0.07*		Total time = 38.9
Prototype interferometer			
20.2.1986	5.84 ± 0.18	441.6	0.19
6.3.1986	5.91 ± 0.28	441.6	0.11
7.3.1986	5.52 ± 0.15	441.6	0.12
7.3.1986	5.62 ± 0.23	441.6	0.16
12.3.1986	5.60 ± 0.20	441.6	0.14
12.3.1986	5.22 ± 0.33	441.6	0.08
Weighted mean = 5.63 ± 0.08		Total time = 0.80	

* Increased to ±0.15 for possible unknown systematic errors¹.

that the increase in the formal error in the earlier measurement from 0.07 to 0.15 arc ms (ref. 1) was unnecessary. This increase was made to allow for possible unknown systematic errors. Such errors in the present work are believed to be small. As a result of their proximity, the two stars were observed at almost identical positions in the sky for a given pair of delay curves. Although

seeing changes with time, its effect was minimized by measuring the two stars in rapid succession, and any residual effect will be largely averaged out by the six independent measurements. It is also worth noting that ~40 h of observations were required with the Narrabri instrument, whereas <1 h was needed to obtain comparable accuracy with the new prototype interferometer.

An extensive observational programme is continuing with the prototype interferometer, with the aims of providing a full understanding of the influence of seeing on its performance and of optimizing its performance for the prevailing seeing conditions.

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The excitation and damping of solar oscillations

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Present theories suggest two classes of excitation processes which may be responsible for the observed amplitudes of solar p-mode oscillations—self-excitation of the modes via an overstability mechanism such as the κ mechanism², and stochastic excitation by turbulent convection^{2–4}. We now have data which stand in support of the latter mechanism. Linear overstability calculations are still ambiguous, because of the uncertainties involved in including turbulent viscous damping of the modes. Nevertheless no calculation predicts overstable f-modes, and these modes are observed on the Sun. Furthermore no nonlinear damping mechanism has yet been proposed which would limit the growth of overstable modes to their observed amplitudes. Assuming the modes are stably damped, the theory of mode excitation by convective turbulence now gives mode amplitudes that are in agreement with observations⁴. In this letter we use data from Big Bear Solar Observatory to compare theory and observation in detail. In particular, our data show that the energy per oscillation mode is nearly constant at low mode frequencies, and is approximately independent of degree at low degrees. The total energy in all the oscillation modes is estimated at $\sim 10^{34}$ erg.

A great deal of attention has recently been given to the problem of comparing calculated and measured frequencies of solar oscillation modes, for the purpose of determining the properties of the interior of the Sun⁵. While the existence of a resonating solar acoustic cavity nicely explains the existence of the observed well-defined normal modes of oscillation, and solar models reproduce the mode frequencies to accuracies of a few per cent, there is still considerable debate about the excitation mechanism that pumps energy into the modes.

Radial oscillations in Cepheid variables are known to be driven by the κ -mechanism, and this was the first mechanism proposed for the excitation of the solar oscillations. Recent mode stability calculations seem to indicate that turbulent viscosity stabilizes the modes⁶; however, the question will doubtless remain inadequately answered theoretically without a better theory of turbulent convection.

Stochastic excitation calculations also rely on our current understanding of turbulent fluid motions, and are therefore also uncertain. However, because the Q (the quality factor) of the oscillations is very high (of the order of 10^3), the coupling between oscillations and convection is necessarily small, and so it is simpler to deal with than for the unstable convective modes. Hence the oscillation modes may prove useful for increasing our understanding of the dynamics of solar convection and the structure of the top of the convection zone.

Because the theoretical calculations of mode excitation are difficult, it is desirable to extract as many clues as possible from the data. The data presented here were acquired at Big Bear Solar Observatory in the summer of 1985, and include two separate data sets at low and high spatial resolution. The first of these consists of 8,042 solar Doppler images which were taken at a rate of one per minute over the 12-day period 22 July–2 August, using the 6,439 Å calcium line, consisting of 200×200 pixels covering the full disk of the Sun. The images were acquired using our new helioseismology telescope which has been described elsewhere⁷. The second data set consists of 3,457 Doppler images taken at the same rate of one per minute over the 5-day period 18–22 August, using the 6,103 Å calcium line, consisting of 512×480 pixels covering a region of the solar disk 266×192 arcseconds in size. These images were acquired using the BBSO videomagnetograph system⁸ configured for Doppler measurements.

Since the solar oscillation modes have a high Q , it is useful to work in terms of the power per mode. If a single mode has a solar surface velocity in the radial direction $v(\theta, \phi) = \text{Re}[AY_l^m(\theta, \phi)e^{i\omega t}]$, then we define the velocity power P_v to be $|A|^2/8\pi$, which is equal to the squared surface velocity associated with that mode averaged over time and the solar surface. Since the oscillatory velocity of a mode varies with height in the solar atmosphere, a physically more meaningful quantity is the energy

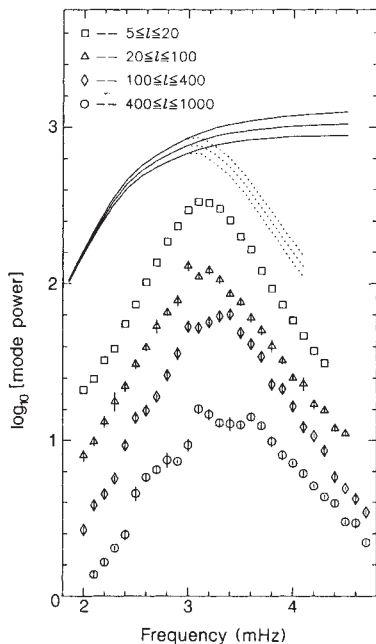


Fig. 1 Mode velocity power P_v , ($\text{cm}^2 \text{s}$) measured as a function of frequency. The data points show the measured power averaged over l for four different ranges of l . Only the lowest- l data are properly normalized, the other three sets of points were arbitrarily scaled; see Fig. 2 for the l -dependence of the power. The solid lines give the scaled logarithm of the inverse mode mass, $[28 - \log_{10} M_v(\text{grams})]$, which is the expected functional form of P_v if the modes all have equal energies. The upper, middle, and lower curves were calculated for optical depths $\tau = 0.02, 0.05, 0.1$ respectively, with $l = 10$. The dotted lines are $[28 - \log_{10}(M_v L)]$, where L is the measured mode linewidth in μHz .

per mode. We define the mode mass $M_v = E_{\text{kinetic}}/P_v$, which is calculated from a solar model given the optical depth of the measurements. Although a detailed solar atmosphere calculation is needed to accurately convert from P_v to energy, for our present purposes it is sufficient to assume that our velocity measurements take place at a fixed optical depth near $\tau_{5,000} = 0.05$ (ref. 9). From our Doppler measurements we are able to obtain the power per mode as a function of l , m , and n , where n is the number of radial nodes in the mode eigenfunction, which we can write as $P_v(\nu, l, m)$ since ν is a monotonically increasing function of n for fixed l and m .

Power versus frequency. The low-resolution data were used to measure the absolute power per mode, since the individual mode peaks could easily be snipped from the data. A total of 8,218 modes were identified with $l \leq 20$, and the power from each was integrated over an 11.6 μHz -wide frequency bin. Various corrections were made to the data to make sure the absolute power per mode was obtained⁷, including the m -overlap described below. The noise in the low-resolution measurements was sampled at $\pm 5.8 \mu\text{Hz}$ from the peak centre, and was found to be small over the frequency range investigated here.

Relative velocity power as a function of frequency was derived from the low- and high-resolution data sets by averaging over l to smooth out the effects of a discrete number of ridges in the l - ν power plot. The results are shown in Fig. 1. Low-resolution data were used for $l < 100$, while the high-resolution data were used for $l > 100$. Since the data were taken using two different solar spectral lines, one expects to see some difference in power due to the different optical depths of the lines. However, in this case the two lines are quite similar, and examination of an overlapping range of l showed that the differences in the power plots are very small. Although there appears to be a general trend of the power peak to become broader and shift to higher ν at higher l , to first order the power can be written as $P_v(\nu, l) \approx P_{\text{max}} N(\nu) \Lambda(l)$, where $N, \Lambda \in [0, 1]$.

If the modes are excited by convective turbulence, then each mode is expected to come into energy equipartition with the kinetic energy of one resonant turbulent eddy³. Assuming the latter energy is roughly constant over the frequency range of interest, then each mode would have roughly the same mean energy, and the mode power would go as M_v^{-1} , shown in Fig. 1. Clearly the modes approach energy equipartition at low ν , but depart from it at frequencies above the power maximum. A much better fit to the data at high ν is obtained by plotting $(M_v L)^{-1}$, where L is the measured linewidth of the modes⁷. Figure 1 shows this result using L measured in μHz , and assuming $L = 1 \mu\text{Hz}$ for $\nu < 3 \text{ mHz}$.

The fit suggests the following phenomenological model: let each mode be excited and damped by energy exchange with convection, where the convective coupling is the same for each mode, and include a secondary damping mechanism, at high frequencies only, with $\dot{E}/E \sim L$. This model gives two power behaviours: at low ν the secondary damping is negligible, the modes interact only with the convective 'heat bath' and so settle the energy equipartition giving $P_v \sim M_v^{-1}$; and at high ν the convective damping is negligible compared to the secondary damping, so setting the constant energy input from convection equal to the secondary damping losses gives $P_v \sim (M_v L)^{-1}$. Possible mechanisms for the high- ν damping include radiative damping or perhaps acoustic energy lost to the upper atmosphere via three-mode coupling.

Power versus spherical harmonic degree. The velocity power as a function of l was obtained using the low- and high-resolution data sets by first fitting the power vs. frequency, $N(\nu)$, using the data in Fig. 1, and then dividing the data by the fit curve and integrating to produce $\Lambda(l) = (P_{\text{max}} \Delta \nu)^{-1} \int P_v(\nu, l) N(\nu)^{-1} d\nu$. The result was also normalized with the mode frequency spacing to produce the relative power per oscillation mode, which is plotted in Fig. 2.

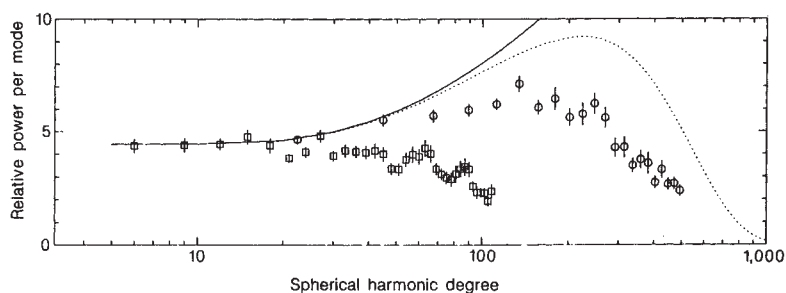
Power as a function of l is difficult to measure due to systematic effects where the overall response of the measurement varies with the scale of the oscillation modes. The two main problems, apparent in Fig. 2, are pixel effects and atmospheric seeing. The former affects the low-resolution data when the pixel size becomes comparable to the spatial wavelength of the oscillations, and severely cuts down the measured power for $l \geq 70$. The high-resolution data were taken with half-arcsecond pixels, so here pixel effects are negligible and atmospheric seeing must eventually degrade the measurements at high l . If the seeing were gaussian with δ arcs resolution (FWHM), the measured power at high l would be diminished by a factor of $\exp(-l^2 \delta^2 / 5.5 R_\odot^2)$. The dotted line in Fig. 2 shows the expected equal-energy power as a function of l with 5-arc s seeing. Unfortunately our Doppler measurements did not include simultaneous measurements of the seeing with the same instrument, so it is difficult to estimate the extent of the errors in the power measurement. Nevertheless it is unlikely that the seeing effects were as bad as that shown in Fig. 2, and so it is unlikely that the data are well represented by the M_v^{-1} curve.

A falloff in mode power at high l compared with energy equipartition could possibly be explained by the same secondary damping mechanism responsible for the power falloff at high ν . We are attempting to measure the mode linewidths at high l -values, which may shed some light on this question, but a better theoretical understanding of this damping mechanism is needed.

Assuming that $P_v(\nu, l) \approx P_{\text{max}} N(\nu) \Lambda(l)$, and incoherently adding the velocity contributions from all the modes up to $l = 1,000$ gives the velocity of a single point on the solar surface to be $\sim 0.2 \text{ km s}^{-1}$, which is comparable with what has been measured.

Power versus spherical harmonic order. If the Sun was spherically symmetrical, the mode power would not depend on m . We measured the power as a function of m in our low-resolution data by fitting for $\Lambda(l)$ and $N(\nu)$, dividing the measured

Fig. 2 Relative mode velocity power P_v as a function of spherical harmonic degree l , averaged over frequencies $2.5 \leq \nu \leq 3.5$ mHz. The squares are from the low-resolution data set, the circles are from the high-resolution data. The solid line is $M_v^{-1}(l)$ for $\tau = 0.05$, scaled using $M_v^{-1}(l=0, \nu)$ and averaged over frequency, which is the expected form of P_v if the modes have equal energies. The dotted line is $M_v^{-1}(l)$, multiplied by a seeing function for 5 arc s seeing (FWHM). The high-resolution data and calculated $M_v^{-1}(l)$ were scaled to match the low-resolution data at low l . The turnover of the low-resolution data for $l \geq 70$ is due to pixel size effects, while the turnover of the high-resolution data is partly due to atmospheric seeing.



$P_v(\nu, l, m)$ by both these functions, and averaging over $5 \leq l \leq 20$ and $2.5 \leq \nu \leq 3.5$ mHz. The low-resolution data set includes fits to all the spherical harmonics in this l -range, but the fits do not completely isolate the different spherical harmonic modes since the back side of the Sun was not observed⁷. However, the mode overlap in the fits is easily calculated, and the measured power vs. m is consistent with the calculated function, shown in Fig. 3. It may be significant that the data here were acquired during a quiet time of the solar activity cycle. It is not inconceivable that when the Sun's active latitudes are covered with sunspots the Sun's spherical symmetry will be sufficiently broken to affect the oscillation mode amplitudes.

With the above data in hand, one would now like to make some statements about the mechanisms that excite and damp the solar oscillation modes. Unfortunately it is difficult to compare self-excitation models with the data, since predicting the amplitudes of self-excited modes depends on specifying an, as yet unknown, nonlinear damping mechanism which limits mode growth. Nevertheless if this damping depended strongly on velocity, which is not unreasonable, then the high-mass modes should have greater energy than those with lower masses; this is not in agreement with the observed energy equipartition of the low-frequency modes. Furthermore, if there were no coupling between the modes, one would expect that self-excited modes with the same l and n but different m would all be excited to a constant amplitude which is independent of time. Inspection of our low-resolution data at $m \approx \pm l$ (where mode overlap in the Y_l^m fitting is unimportant) shows that many modes had very small amplitudes during the 12-day run, which is more consistent with a Boltzmann-type distribution. Thus it seems that the data do rule out a self-excitation model where the modes are independently damped (that is, no mode coupling). However a strong

nonlinear coupling between self-excited modes may reproduce the observations.

The simple phenomenological model described above, based on the convective turbulence excitation model, fits the observations to lowest order, but there are still substantial discrepancies. The modes have nearly a constant mean energy at low frequencies, but, as shown in Fig. 4, the energy per mode shows a strong peak near 3 mHz. This suggests there is either a stronger excitation or an anomalously low damping at the power maximum; the latter should show up in better mode linewidth measurements. Also the power as a function of l does not exactly fit the convective turbulence model, since the model predicts energy equipartition for modes with different l (ref. 4). However, this may be because we do not yet know the magnitude of the secondary damping (or the mode linewidths) at the higher l values.

At low l and low ν the modes are nearly in energy equipartition, and as shown in Fig. 4 the mean energy per mode goes to $\sim 3 \times 10^{27}$ erg. At the power maximum for low l the energy per mode is three times as great, and only above $\nu = 3.8$ mHz does the energy drop below this value. Assuming all modes with $\nu < 2$ mHz have a mean energy of 3×10^{27} erg, the total energy in all the $l = 10$ modes is 2×10^{30} erg. At $l = 50$, the mode spacing in ν has increased, and there are fewer modes with a given l and m having $\nu < 2$ mHz, but there are more possible m values, so the total power in all the $l = 50$ modes is $\sim 8 \times 10^{30}$ erg. At higher l the mode spacing in frequency increases further, and if we assume P_v is roughly constant with l , then the energy per mode drops like $l^{-1/2}$ since $M_v \sim l^{-1/2}$. But these two factors are nearly cancelled by the increased number of possible m values, so the total power in all the modes having a given $l \leq 500$ is comparable with the $l = 50$ value. Adding up all the

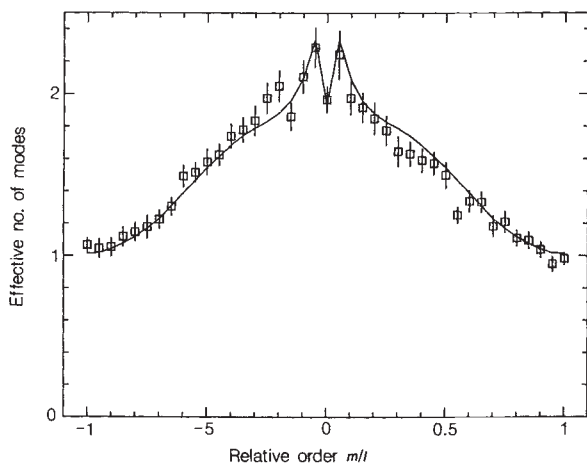


Fig. 3 Relative mode velocity power as a function of spherical harmonic order m , averaged over all modes with $5 \leq l \leq 20$. The curve is the calculated effective number of modes with the same l and n but different m included in a single $Y_l^m(\theta, \phi)$ fit, also averaged over all modes with $5 \leq l \leq 20$. The data were scaled to agree with the calculated curve. The very good fit indicates that the data are consistent with there being no intrinsic m -dependence in the mode power.

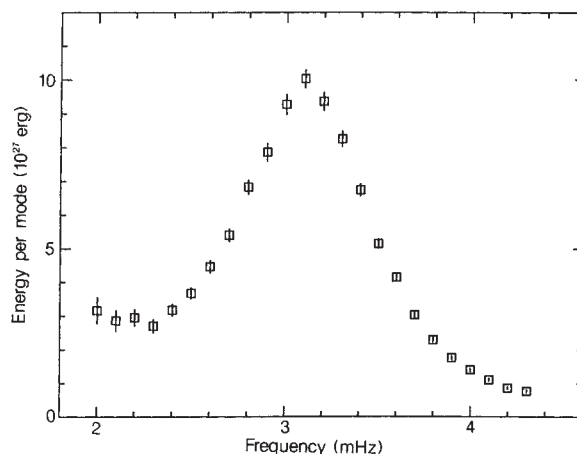


Fig. 4 Energy per oscillation mode as a function of mode frequency, averaged over modes with $5 \leq l \leq 20$, and assuming the mass function calculated for $\tau = 0.05$. The error bars reflect the errors in the relative energy distribution; other normalization errors could shift the absolute value of the energy measurement by as much as 25%. Note that the high-frequency end of the plot is most affected by assuming an incorrect optical depth for the measurements.

observed oscillation modes gives a total oscillation energy of $\sim 10^{34}$ erg, although this number could be in error by as much as an order of magnitude.

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Rossby autosoliton and stationary model of the jovian Great Red Spot

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A theory proposed about 10 years ago claimed that the jovian Great Red Spot (GRS) was a solitary wave vortex (Rossby soliton) kept stationary by counter-streaming zonal winds. We have attempted to verify this soliton theory experimentally. The jovian atmosphere is modelled by a rotating thin parabolic layer of fluid (shallow water) with a free surface in which counter-streaming (zonal) flows are excited mechanically. We have found that instability of these flows can generate a Rossby autosoliton, that is, an undamped stationary solitary vortex which is alone on the perimeter of the system. This vortex rotates around its axis in the anticyclonic sense and drifts in the opposite direction to the global rotation of the system. As the observed Rossby soliton can be considered as a physical analogue (or rather as a stationary physical model) of a natural vortex such as the GRS, the results of our experiments can be considered to support the soliton theory of the GRS. We have compared the soliton model with another physical model of the GRS based on thermoconvection in rotating deep water under the action of a transverse non-monotonic temperature gradient. A new model based on a synthesis of these ideas is urgently required.

In our experiments carried out on a hydrodynamical model of the homogeneous atmosphere of a rotating planet^{1–3} we have created and investigated for the first time the Rossby soliton, that is, an anticyclonic solitary vortex in a rotating parabolic layer of shallow water which has a free surface. The vortex has a characteristic diameter of a few Rossby–Obukhov radii (ref. 1) and drifts counter to the rotation of the system as a whole, keeping its shape unchanged. (The possibility that such a soliton exists has already been pointed out⁴.) The physical characteristics and the regularities of propagation of the Rossby soliton generated in experiments^{1–3} are similar to a natural vortex such as the GRS, and can be considered to be a laboratory model of the latter, thus qualitatively demonstrating the soliton theory of this natural phenomenon^{4–7}. However, there are shortcomings in this version of the model, in that the vortex has been excited by a localized source of short duration and has had a comparatively small lifetime limited by the characteristic time of viscous damping, $\tau \approx H_0^2/\nu$, where H_0 is the thickness of the fluid layer and ν is the kinematic viscosity. The natural vortex GRS is, however, stationary, being 'fed' by zonal flows^{5,6}. The latter are counter-streaming winds blowing along parallels, and their amplitude and direction depend on the latitude angle. Such a picture of zonal flows is a result of the evolution of two-

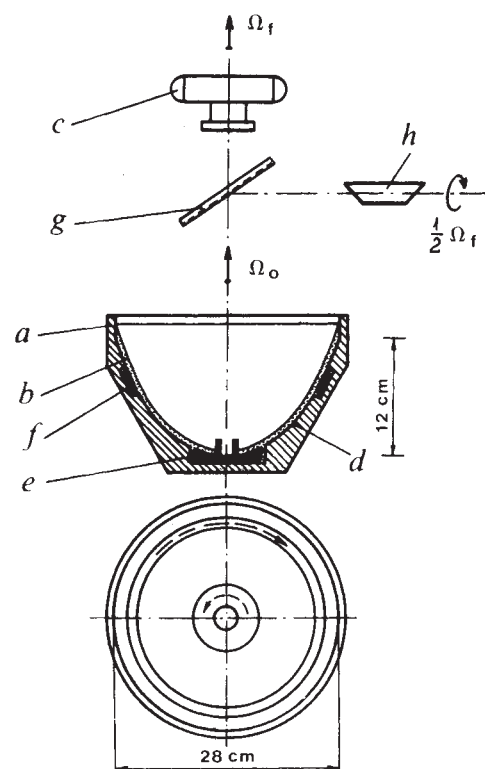


Fig. 1 Experimental set-up in which the Rossby autosoliton was first produced. *a*, a rotating vessel with a parabolic bottom (diameter of the vessel is 28 cm); *b*, surface of water; *c*, camera rotating together with the observed vortex; *d*, gap between flows ($L=11$ cm); *e*, section of the paraboloid bottom which rotates faster than the vessel; *f*, a section of the paraboloid bottom which rotates slower than the vessel; *g*, semi-transparent mirror; *h*, roto-scope (based on the rotating Dove prism, making it possible for an observer to transfer himself to the rotating reference frame). The vessel rotates counterclockwise. $\Omega_0 = 15 \text{ s}^{-1}$ is the vessel rotation frequency; Ω_f is the camera rotation frequency.

dimensional (barotropic) turbulence in the atmosphere of a rotating planet^{8–11}. To ensure that the soliton model under consideration has similar properties to a natural vortex such as the GRS, an experiment was carried out in which excitation of a Rossby soliton, that is, a solitary vortex which is the only one on the whole perimeter of the system, was produced without any local source, but due only to the counter-streaming zonal flows generated mechanically.

In our experiment (Fig. 1), the shape of the bottom of the vessel allows the fluid poured into it to be distributed as a layer of constant depth under rotation with a certain velocity. The diameter of the 'paraboloid' is 28 cm; its height is 12 cm. The working fluid (water), distributed, when rotated, in a thin layer over the bottom of the vessel, is subjected to the action of counter-streaming flows propagated along the parallels around the axis of system. The flows within the rotating vessel are generated by the movement of two sections of its bottom which rotate in opposite directions with respect to the vessel and involve the layers of fluid located above. The internal movable section of the bottom has a diameter of 10 cm. The external section is a ring of width 2.5 cm. It is situated at a distance $L=11$ cm from the internal section (both of these dimensions are measured along a meridian of the paraboloid). In the gap between the two movable sections is a zone of a 'proper' paraboloid rotating as a whole. The working thickness of the fluid layer is $H_0=0.5\text{--}1$ cm. The flows and structures formed are visualized using white probe particles (small pieces of white paper), which float on the surface of the water and show up the black bottom of the vessel. They are observed through a roto-scope which is based on the Dove prism. The rotation of the

camera around the axis of system is synchronized with the observed drift of the vortex. The main results of the experiment are as follows.

At a sufficiently high relative velocity, the counter-streaming flows exhibit instability. This is manifested by the generation of a chain of m anticyclonic vortices drifting along the parallel situated between flows.

Under a given geometry of flows and frequency of paraboloid rotation, the main parameter governing the instability is the relative velocity of flows. With a certain flow velocity a structure with only one vortex on the perimeter of the system is formed ($m = 1$). This result will be discussed later.

Vortices excited by the unstable flows are rather localized solitary formations. Their characteristic size (diameter) is 3–4 r_R , where

$$r_R = (g^* H_0)^{1/2} / 2\Omega_0 \cos \alpha \quad (1)$$

is the Rossby–Obukhov radius; Ω_0 is the angular velocity of rotation of the system as a whole; g^* is the resultant of the accelerations due to gravity and to the centrifugal force from the global rotation; α is the angle between the axis of system rotation and the local normal to the equilibrium surface of the fluid in the absence of flows.

Vortices are characterized by the Rossby regime; that is, the frequency of the proper rotation of the vortex is $< \Omega_0$. An approximately geostrophic equilibrium obtains between the Coriolis force, acting on a circular current of particles inside the vortex, and the gradient of the hydrostatic pressure.

Vortices drift in the opposite direction to the paraboloid rotation; that is, they lag the system rotation as a whole. The velocity of vortex drift is approximately equal to the Rossby velocity V_R . In a parabolic vessel with $H_0 = \text{constant}$ ^{1,2}, the latter is given by

$$V_R = \frac{1}{2} H_0 \Omega_0 \sin \alpha \quad (2)$$

The maximal velocity of particle rotation in the vortices exceeds their drift velocity; therefore, vortices effectively capture the particles involved.

By varying the main parameters and geometry of flows using three modifications of the installation ($L = 0.1, 3$ and 11 cm), we have shown that the relative velocity of the flows which is necessary for excitation of a chain of vortices of a given azimuthal mode m , grows with the increase of Ω_0 , H_0 and L . This corresponds to the following relation: a difference in velocities of flows over a size of vortex (Ua/L) should provide a speed of vortex rotation significantly exceeding the drift velocity $\sim V_R$, because just in this case we observe a vortex with a clear-cut capture region (Fig. 2). The indicated relation (at $L \gg H$) is of the form

$$u \approx c V_R L / r_R \approx c \Omega_0^2 L (H_0 / g^*)^{1/2} \quad (3)$$

where the coefficient c is of the order of a few units and depends on the experimental details.

If the distance between the counter-streaming flows is large enough ($L = 3$ or 11 cm), their instability generates large-scale vortex structures (with characteristic size $> r_R$) only for an anticyclonic direction of flows, when their curl is antiparallel to Ω_0 ; when the curl of the flows is cyclonic, then large-scale vortex structures are not generated. This cyclonic–anticyclonic asymmetry is a fundamental nonlinear feature of the Rossby vortices. It occurs because large-scale anticyclones can be the long-lived Rossby solitons, but the large-scale cyclones cannot because, in contrast to anticyclones, the nonlinearity of cyclones does not compensate for a dispersion decay of the wave packet^{3,11}.

The most interesting structure obtained is the solitary vortex at a mode $m = 1$, which is unique over the whole perimeter of the system (Fig. 2). The vortex is an anticyclone of characteristic diameter 3–4 r_R , drifting ‘westwards’ with a velocity of $\sim 8 \text{ cm s}^{-1} \approx V_R$. We have identified it as a Rossby soliton^{1–3}.

Figure 3 shows the profiles of fluid depth and velocity in the

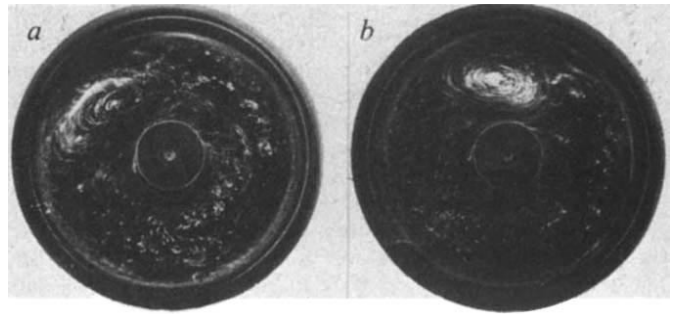


Fig. 2 *a, b* Two realizations of the Rossby autosoliton, mode $m = 1$, in similar experimental conditions. $\Omega_0 = 15 \text{ s}^{-1}$; camera exposure, $1/3 \text{ s}$. The distance from the vortex centre to the rotation axis is $\sim 6 \text{ cm}$.

vortex (Fig. 2) and in surrounding flows. The vortex arising through the instability of counter-streaming axially symmetric flows rearranges their profile completely: if before the vortex emerges the flow velocity profile has the form of a step function (*a*), then in the regime which contains the vortex, the profile is a smooth curve (*b*). The velocity profile inside the vortex is depicted by two curves (*c*) in Fig. 3A,B. The flows depicted by the new profiles are consistent with the spatial structure of the vortex. They generate the vortex and compensate for its viscous and, probably, other losses. The maximum velocity of the vortex rotation is $\sim 15 \text{ cm s}^{-1}$, significantly greater than the drift velocity

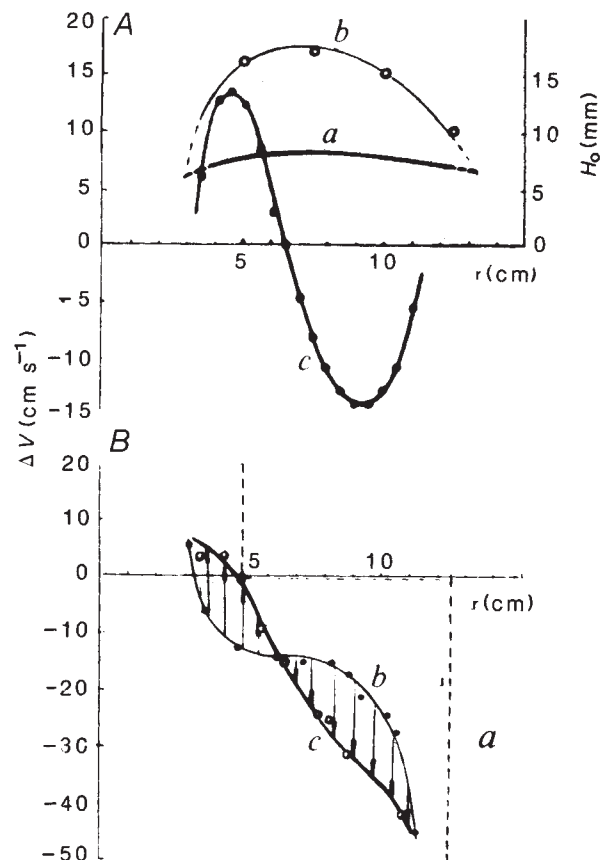


Fig. 3 *A*, Profiles of the fluid layer thickness (H_0) and velocity (Δv) in the meridional cross-section of the rotating paraboloid. *a, b*, Dependence of the layer thickness on distance to the axis of rotation (r): *a*, in the region diametrically opposed to the vortex, and *b*, in the vortex region. *c*, Velocity profile inside the vortex (relative to a flow—see arrows in *B*). *B*, Profiles of linear azimuthal velocity of particles on a fluid surface in the rotating vessel reference frame: velocity versus distance to the vessel axis. *a*, In the absence of a vortex; *b*, in the regime of a vortex driven in the region opposite to the vortex; *c*, inside the vortex.

of the vortex, and corresponds to the presence of the pronounced region of captured particles within the vortex (Fig. 2). The maximum vorticity (curl of the vortex) exceeds the curl of the flow outside the vortex (on the opposite side of the vessel) by a factor of 5–6, similar to the case of large-scale vortices in the atmospheres of Jupiter and Saturn^{12,13}.

The $m=1$ vortex exists for an infinitely long period of time, keeping its shape without significant changes.

Thus, the Rossby $m=1$ vortex generated in our experiment has all the characteristic features of a Rossby soliton and exists for an infinitely long period of time; that is, it is a self-sustained Rossby autosoliton. As for the possibility of an instability mechanism generating a Rossby soliton, there are some grounds for considering it as a centrifugal instability of a differentially rotating fluid, the inner part of which rotates faster than its periphery (this corresponds to an anticyclonic curl of the counter-streaming flows). The Rossby autosoliton results from the nonlinear self-organization of a Rossby soliton in the zonal flows, and can be considered as a realization of the stationary laboratory model of the GRS, supporting the soliton theory^{4–7} of this natural phenomenon. Certainly, such a two-dimensional (barotropic) model is an inadequate description of the wave motion of a real natural vortex such as the GRS, which seems to be three-dimensional. However, the vertical wave motion in the vortex (baroclinicity) can be easily accounted for. This allows us to use the two-dimensional (simple) model to simulate qualitatively the natural vortices under study. When considering the problem, quantitatively, it is easy to make the necessary corrections for three-dimensionality, as described in ref. 7. A similar approach has been used in the barotropic theories^{8–11} of planetary atmosphere circulation which result in an adequate and generally accepted qualitative understanding of the mechanism of zonal flow formation in the planetary atmospheres.

The soliton model of the GRS and one of its most important components—the cyclonic–anticyclonic asymmetry—agrees well with the fact that all large, long-lived vortices in the jovian and saturnian atmospheres are anticyclones, with the exception of cyclonic ‘barges’ in the jovian northern hemisphere. According to our concept, this exception can be simply explained because the cyclonic barges are the result of extremely strong ‘pumping’ by unstable cyclonic flows with extremely steep meridional velocity gradients (much steeper than in the GRS⁷). In flows containing such steep gradients, the growth rate of the instability which generates vortices considerably exceeds the decay rate of cyclones due to dispersion. This explanation is founded on experimental data obtained when the experimental installation is operated with $L=0.1$ cm; that is, the gap between the counter-streaming flows is ≈ 1 cm. Our experiments have shown that in this case the (unstable) flows with a cyclonic curl generate a chain of large-scale stationary cyclones, similar to that of anticyclones. These cyclones, like the jovian barges, drift to the east (see also ref. 11).

Our experiments differ from other investigations of the geostrophic structures in some essentials. No previous experiment has combined such important conditions as: (1) shallow water with free surface; (2) β -effect; that is, vortex drift counter-rotation of the system as a whole due to the dispersion of Rossby waves; (3) differential rotation; and (4) large scales of vortices ($a > r_R$).

However, it is this combination which leads to such interesting structures as the Rossby autosoliton and such attractive non-linear phenomena as cyclonic–anticyclonic asymmetry. As examples of other investigations of geostrophic structures, one can point out interesting experiments^{14,15} which do not meet conditions (1), (2) and (4) above.

Now let us compare our soliton model with another model of the global atmospheric vortices¹⁶ which is based on thermoconvection in a rotating fluid and has the following features which distinguish it from our model. (1) It is essentially baroclinic and leads to the realistic conclusion that the size of the

natural vortices must be determined by the baroclinic Rossby radius, r_i . [In our barocline modification⁷ of the considered barotropic soliton model, the substitution of the barotropic Rossby radius r_R for the baroclinic radius r_i is also carried out.] (2) It uses a water depth which exceeds (by many times) the diameter of the vortices under consideration and in this sense corresponds to a theoretical concept^{17,18}. (3) It does not take into account the dispersion effects and does not explain the drift velocities of the natural vortices. (4) It does not imply the cyclonic–anticyclonic asymmetry of long-lived large vortices observed on Jupiter and Saturn.

A new experimental model unifying both theories is needed, which would take into account both baroclinity and vortex drift and cyclonic–anticyclonic asymmetry and give a natural explanation of such exceptions as the cyclonic jovian ‘barges’.

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Product yield of magnetic-field-dependent photochemical reaction modulated by electron spin resonance

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Magnetic-field-dependent yields in many chemical reactions^{1–13} have been interpreted by the so-called ‘radical pair’ model^{14–16}. To establish the mechanism of this dependence, we have measured the product yield of a magnetic-field-dependent photochemical reaction under a magnetic field by spin-trapping a radical intermediate and using electron spin resonance (ESR). On irradiating with microwave and ultraviolet light simultaneously under various magnetic fields, the ESR spectra of the free radicals composing the radical pair can be traced by measuring the spin-adduct yield. This observation, which we term ‘product-yield-detected ESR’, provides direct evidence that radical-pair interaction modifies the reaction yield. This is the first experiment which has demonstrated that an ESR transition can modify the product yield.

The radical pair model assumes three steps in the magnetic-field-dependent reaction: (1) during the course of the reaction, a pair of free-radical intermediates are formed; (2) the transition frequency between the two possible states of the radical pair, singlet and triplet, is influenced by their nuclear spin states and the external magnetic field; (3) only the singlet radical pair can react to yield the so-called ‘cage product’. To investigate this

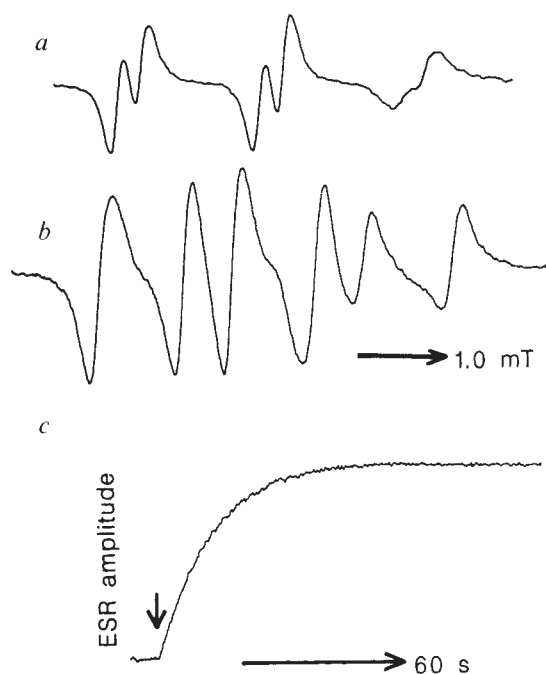


Fig. 1 ESR spectra and time path of the signal of the spin adducts in the photo-reduction of menadione in SDS micellar solution: *a*, with PBN; *b*, with DMNS as the spin trap; *c*, time path of the signal observed at the lowest-field peak of spectrum *a*. The arrow indicates the time when ultraviolet irradiation started. The reactant solution contains 0.1 mM menadione and 0.4 M SDS. The solvent used was a phosphate-buffered saline (90 mM NaCl, 50 mM Naphosphate, pH 7.4). Either PBN or DMNS was added as a spin trap at the final concentration of 17 mM and 1.0 mM, respectively. Photo-irradiation was performed as described previously³: the light from a 500-W ultra-high-pressure mercury lamp (Ushio UH-500D, Tokyo), filtered by aqueous CuSO_4 solution (1.0 cm, 1.2 M), was used to irradiate the de-aerated sample solution for 1.0 min at $23 \pm 1^\circ\text{C}$, in a flat ESR cell placed in a cavity resonator (for *in situ* photolysis) of a Varian E-3 ESR spectrometer. The modulation field and the microwave power were 0.1 mT and 5 mW, respectively.

model, we spin-trapped a radical intermediate in the photo-reduction of menadione (2-methyl-1,4-naphthoquinone) in a micellar solution of sodium dodecyl sulphate (SDS) under a magnetic field in the presence and absence of a microwave field. The magnetic-field-dependent kinetics of the semiquinone radical of menadione in this system have already been discussed by Sakaguchi and Hayashi². The spin trapping technique¹⁷ is advantageous for this kind of study, because stable spin adduct, formed by the reaction between transient free radicals and the spin trap, can accumulate and the yield of the spin adducts can be measured following irradiation by ultraviolet light and a microwave field. The product-yield-detected ESR, thus obtained, was the superposition of the ESR spectra of the SDS radical and the protonated semiquinone radical, which have been proposed to form the radical pair^{2,3}. This is direct evidence for the model mentioned above and also supports the radical pair theory of chemically induced nuclear/electron spin polarization phenomena^{18,19}.

Figure 1 shows the ESR spectra of the spin adducts obtained using two spin traps, phenyl-*tert*-butylnitron (PBN; spectrum *a*) and perdeuterio-2,4-dimethyl-3-nitrosobenzenesulphonate²⁰ (DMNS; spectrum *b*), and the time path of the increasing ESR signal during the photo-reduction of menadione (*c*). Both spectra were the same as those assigned to the spin adducts of SDS radical reported previously³.

Figure 2 shows the magnetic field dependence (without microwave irradiation) of the spin-adduct yield obtained with PBN (curve *a*) and DMNS (curve *b*). In both cases, the yield increases

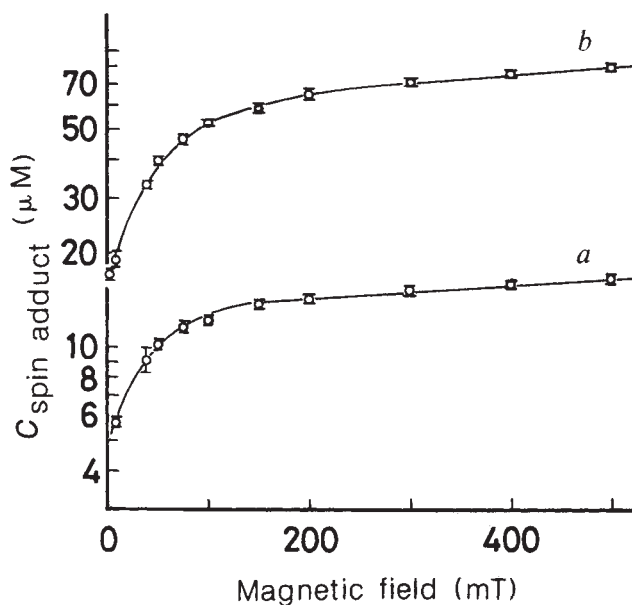
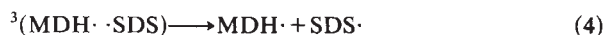


Fig. 2 Magnetic field dependence of the yield of the spin adduct of the SDS radical during the photo-reduction of menadione in SDS micellar solution with PBN (*a*) and DMNS (*b*) as the spin traps. The experimental conditions were as described in Fig. 1. The observations were made 1.0 min after the photo-irradiation and were repeated three times under various magnetic fields in a random order. The average values and standard errors are shown. The concentrations of the spin adduct were determined by doubly integrating the spectra and comparing them with that of purified TEMPO (2,2,5,5-tetramethyl-piperidine-1-oxyl) of a known concentration.

with the increase of the magnetic field under which the photo-irradiation occurs. Because no magnetic field dependence is expected in the spin trapping process itself, the yield of the escaped SDS radical must depend on the magnetic field in the way shown in Fig. 2. The reaction scheme based on the radical pair model which explains these magnetic-field-dependent yields may be²:



Menadione, which is insoluble in water and thus solubilized in the micelle, is excited to its singlet excited state by the ultraviolet light, and part of the excited menadione is converted to its lowest triplet state (equation (1)). Next, the excited menadione in the triplet state abstracts a hydrogen atom from a SDS molecule and the triplet radical pair is formed. The triplet radical pair is converted to the singlet radical pair (equation (2)) and the cage product is formed through the singlet radical pair (equation (3)). Part of the radical pair (mainly in the triplet state) is dissociated and the component radicals escape from the micelle (equation (4)). Equation (5) shows the formation of the spin adduct ($\text{T}(\text{SDS})\text{NO}\cdot$) (the product analysed; see Fig. 1) by the reaction between the spin trap (TNO), which is mainly in aqueous bulk phase (especially for DMNS), and the escaped SDS radical.

At zero magnetic field, the triplet sublevels (T_1 , T_0 and T_{-1}) of the radical pair degenerate and can mix to the singlet state (S_0) due to the hyperfine coupling (hfc). On the other hand, at higher fields two triplet sublevels T_{-1} and T_{+1} are separated

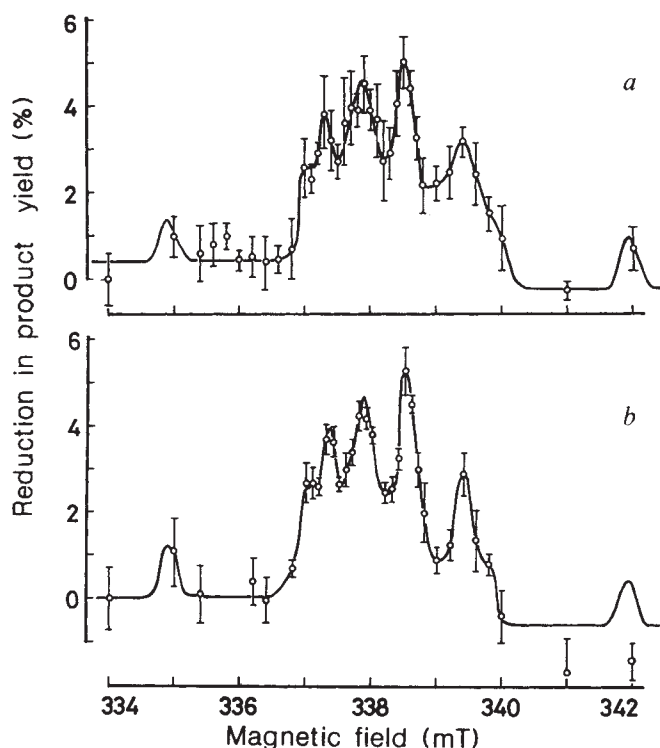


Fig. 3 Reduction in the yield of the spin adduct during photo-reduction of menadione in SDS micellar solution (de-aerated) as a function of the static magnetic field (product-yield-detected ESR), due to the simultaneous irradiation by X-band microwave. The reactant solution was menadione (0.3 mM) and a spin trap, dissolved in SDS micellar solution (0.4 M) in the phosphate-buffered saline. PBN (a) (17 mM) or DMNS (b) (1.0 mM) was used as the spin trap. Experiments were repeated twice, and the change in the spin adduct yield was calculated from the two peaks (lower field) of each ESR spectrum. The averaged values and the standard errors shown were calculated from those four values for each point. Photo-irradiation (500-W ultra-high-pressure mercury lamp) and microwave irradiation (9,488 MHz, 160 mW at the inlet of the cavity) were performed simultaneously for 30 s at $23 \pm 1^\circ\text{C}$. ESR observations were made 1.0 min after irradiation.

energetically from the singlet level due to the electron Zeeman interaction and only the mixing between T_0 and S_0 is allowed (if the exchange interaction between the two radicals is smaller than the hfc). Therefore, if k_p in equation (3) is much larger than k_{ISC} in equation (2), the concentration of the escaped radical ($\text{SDS}^\cdot$) and thus the yield of the spin adduct increases at higher field.

To investigate the above-mentioned model further, we observed the effect of ESR transition on the spin-adduct yield. The ultraviolet light and the X-band microwave were used to irradiate simultaneously the de-aerated sample solution. After a specified waiting time, an ESR spectrum of the spin adduct was recorded. The sample solution was introduced anaerobically into a quartz flat cell placed in the ESR cavity. The experiments were repeated with the new solution using a different magnetic field, which was changed point by point to scan the field. The product-yield-detected ESR spectra, shown in Fig. 3, were obtained by plotting the reduction in the spin-adduct yield due to the microwave irradiation as the function of the magnetic field.

The spin-adduct yield of SDS radical decreased on the simultaneous microwave irradiation at particular magnetic fields, and the product-yield-detected ESR spectra show four distinct peaks in Fig. 3, which are easily assigned to the protonated semiquinone radical of menadione and SDS radical by the simulation spectrum shown in Fig. 4. This decrease can be explained such that the microwave irradiation induces the transitions from $T_{\pm 1}$ to both S_0 and T_0 and increases the effective k_{ISC} in equation (2).

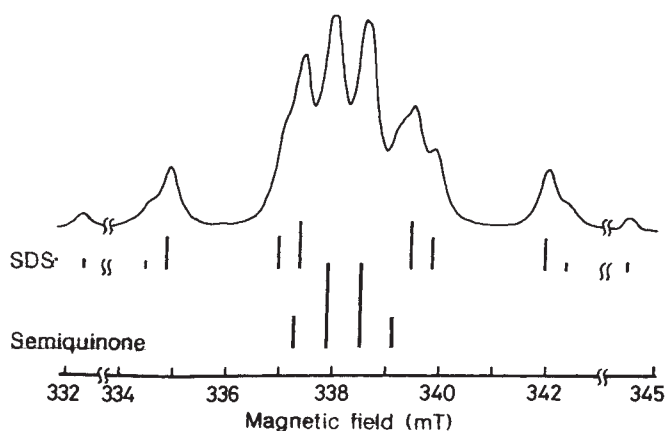


Fig. 4 Calculated ESR spectra for the 1:1 mixture of SDS radical and the protonated semiquinone from menadione. The stick diagrams under the simulated spectrum indicate the positions of the main ESR peaks for the component radicals. The parameters used for the SDS radical²¹ were: $g = 2.0034$; $a_H(\alpha) = 2.1$ mT; $a_H(\beta) = 2.5$ mT; line-width, 0.2 mT; and for the protonated semiquinone radical from menadione²²: $g = 2.0046$; $a_H(\text{methyl}) = 0.63$ mT; $a_H(5, 7, \text{OH}) = 0.155$ mT; $a_H(3) = 0.059$ mT; line-width = 0.1 mT, where g represents the g -factor of the unpaired electron and a_H is the hfc constant of the hydrogen nucleus whose position is specified in parentheses. The hfc of the rest of the hydrogen atoms were taken into account in the line-width parameters. The hfc of methyl hydrogen and the g values were adjusted slightly from the values given in the literature^{21,22}.

These observations have some important implications:

(1) Although we observed only the ESR of the spin adduct of the SDS radical, the product-yield-detected ESR spectrum shows the ESR pattern of both the protonated semiquinone and the SDS radical. This indicates that the yield of the escaped SDS radical is determined by the spin correlation between the component radicals of the radical pair. This provides direct evidence that a radical pair is formed and that the spin dynamics in the radical pair determine the escaped-radical yield.

(2) The exchange interaction and the dipolar interaction between the two spins are quite small even though they form a radical pair, because the line-width of the product-yield-detected ESR spectrum is small (≤ 0.2 mT) and there is little difference between the hyperfine pattern²¹ and those reported elsewhere^{22,23}.

(3) The application of our method to a radical-pair-forming reaction may enable us to control the reaction pathway by the ESR transition.

(4) This modulation of the product yield due to ESR transition is nuclear-spin-selective, because a particular hyperfine component of the ESR transition is irradiated at a magnetic field, as shown in Fig. 3. Thus, populations in the energy levels for the nuclear spins of the products may be strongly polarized, and ESR-induced CIDNP (chemically induced dynamic nuclear polarization) may be possible. In addition, product-yield modulation by ENDOR (electron nuclear double resonance) or product-yield-detected ENDOR may also be observable in this system.

A method, which observes ESR of transient species has also been demonstrated and termed "reaction yield detected magnetic resonance"²⁴. However, although in these studies the fluorescence or photoconductivity of a solid sample is monitored using irradiating light and modulated microwave at high magnetic field, the product yield, as far as we know, has not been measured.

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Estimation of oceanic eddy transports from satellite altimetry

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Ocean eddies, like weather systems in the atmosphere, are an important mechanism for the transport of heat, momentum and matter. The difficulty and expense of observing ocean eddies have prevented the adequate inclusion of eddy effects in studies of global ocean-atmosphere climate. Here I suggest a novel approach, which uses fluctuations in sea-surface elevation, determined from Seasat altimetry, to obtain a simple estimate of eddy transports. The method is applied here to heat and salt transports in the North Pacific. Refinements of theory and modelling, together with data from future altimetric satellites, will improve the precision of the method.

The contribution of the ocean to poleward transport of heat is a major concern of climate studies. A ubiquitous field of energetic ocean eddies frustrates efforts to observe this oceanic transport directly. Calculations using the geostrophic section method¹⁻³ may be unreliable with respect to a spatially and temporally varying velocity reference level. A method is now outlined for estimating the eddy transport using theory and numerical modelling of geostrophic turbulence⁴, together with results from satellite altimetry⁵.

Satellite altimetry can be used to determine the circulation of the upper ocean by observing the elevation of the sea surface relative to a geopotential (geoid) surface⁶, but a major source of error at length scales of hundreds of kilometres is the uncertainty in the geoid. Because it is steady on oceanic timescales, uncertainty in the geoid can be ignored if one considers only the time-fluctuating sea-surface elevation, after correcting for ocean and Earth tides and for atmospheric and sea-state effects. This method, based on 24 days of repeat track altimetry from Seasat, has been used to produce a global map of root-mean-square (r.m.s.) fluctuations of the sea surface⁵. Regions of large elevation variability were found to agree with regions of observed eddy intensity (see Fig. 1a).

Repeat track altimetry can be used to calculate the variation in the tilt of the sea surface, from which can be estimated⁷ r.m.s. surface geostrophic currents or eddy kinetic energy. However, there is a tendency for spatial derivatives to exaggerate errors, and the relation of eddy kinetic energy to eddy transport is unclear. An alternative method, which is less subject to error and is more directly applicable to eddy transport, is based on the r.m.s. geostrophic stream-function, ψ . This is given by $\psi = gh/f$, where g is the acceleration of gravity, h is the r.m.s. elevation fluctuation after removing tidal effects, and $f = 2\Omega \sin \phi$ (where Ω is the Earth's rotation rate and ϕ is the latitude) is the Coriolis parameter.

The relation of ψ to the eddy transport is surprisingly simple. If one considers the idealized dynamics of statistically homogeneous and isotropic two-dimensional turbulence, then an eddy diffusivity K for a passive tracer can be estimated as

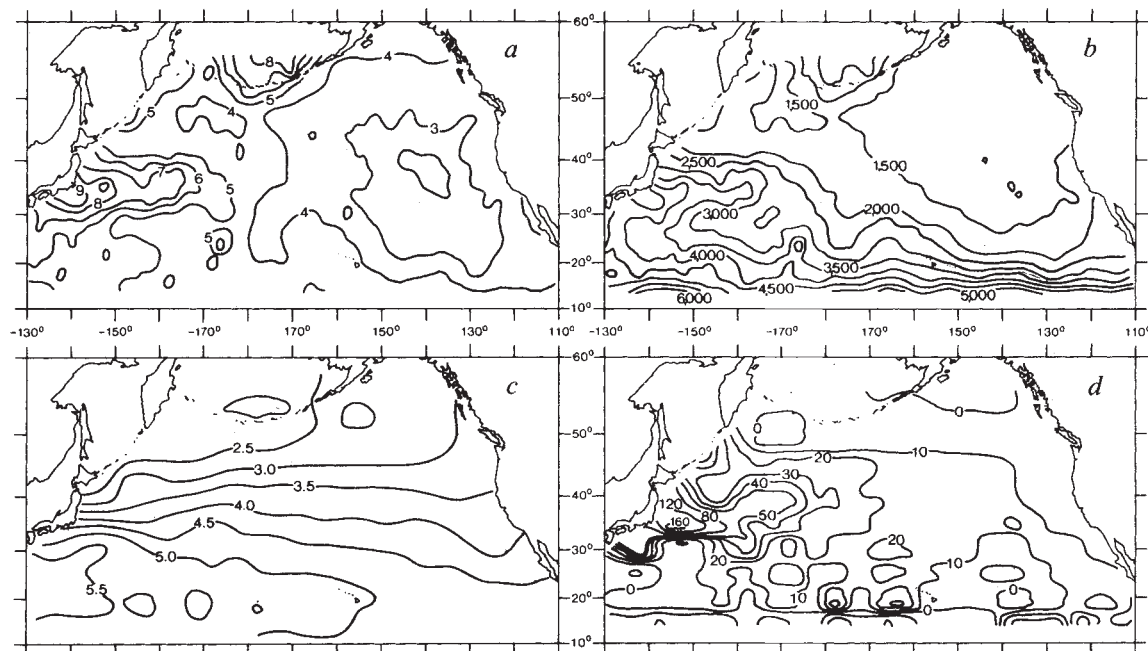


Fig. 1 Contour plots of: a, r.m.s. variation of sea-surface deviation (cm), based on Seasat repeat track altimetry; b, depth-averaged meridional component (K_y) of eddy diffusivity ($\text{m}^2 \text{s}^{-1}$); c, depth-averaged temperature ($^{\circ}\text{C}$); d, depth-integrated northward eddy heat transport (in MW per lineal metre).

$K = C\psi$ with C a numerical coefficient equal to ~ 0.4 . While this result is obtained from detailed closure theoretical arguments⁴, a heuristic motivation can be seen by writing $\psi = ul$, where u is the r.m.s. eddy flow speed and l (defined by ψ/u) is a characteristic eddy length scale which could be a 'mixing length'. The value of C is largely empirical.

The idealization of two-dimensional turbulence has wider applications. Rossby wave propagation can induce anisotropy in the eddy diffusivity tensor $\mathbf{K}$. The meridional component (K_y) of diffusivity will be suppressed⁴ as $K_y = C\psi(1 + (\mu\beta)^2)^{-1}$, where $\beta = \partial f / \partial y$, β is the meridional derivative of f , and ζ is the r.m.s. eddy vorticity. μ is a factor which depends on details of the form of the eddy spectrum. Rossby wave propagation also induces anisotropy in the eddy energetics, suppressing meridional velocity variance relative to zonal velocity variance^{8,9}, thereby further suppressing meridional diffusivity.

There are insufficient altimetric data from Seasat to resolve anisotropy of eddy energy or to provide reliable details of the eddy spectrum. I therefore use numerical models of Rossby wave turbulence, augmenting the spectral form factor μ to reflect also the energetic anisotropy in an effective μ equal to ~ 3 . Seasat altimetry is also unable to determine β , which tends to be a constant^{8,9} in a variety of environments; here I adopt a value of $\sim 1/3$. Whereas altimetry yields ψ at the sea surface, we need to determine r.m.s. ψ distributed over depth. We are also concerned with eddy transports of heat and salt, which, through their effects on density, are dynamically active fields. Much of the variation of ψ at the sea surface is due to a vertically integrated density anomaly (that is, dynamic height). Upper ocean velocities as derivatives of ψ are therefore inefficient at transporting heat. Thus, the methods used to estimate baroclinic eddy transport from hydrographic data¹⁰ are very sensitive to error.

Two assumptions must be made. First, of the total variance of ψ observed at the sea surface, about half is intensified near the surface and half is distributed over the depth of the water column. Only the latter half will be used in the calculation below. Second, meridional transport depends on the meridional

gradient of depth-averaged temperature. The distribution of ψ variance may be more strongly surface-intensified than I suppose; on the other hand, my discounting of upper-ocean transport may be too severe. The present calculations should be viewed as illustrative rather than definitive.

Finally, a correction is necessary for the 24-day duration of Seasat repeat track altimetry. Most oceanic eddy variance is of longer period, and thus is not sampled. By comparison with frequency spectra from long-term moorings and with previous GEOS 3 altimetry, it is estimated⁷ that the 24-day Seasat altimetry sampled only 10% of the total variance. Adopting all these estimates, numerical values and assumptions, a map of depth-averaged K_y was calculated from the Seasat r.m.s. height fluctuation, h (see Fig. 1b).

From atlas data¹¹, a map of temperature, averaged to a depth of 3,000 m, is shown in Fig. 1c. Meridional temperature gradients at depths below 3,000 m are weak and data are sparse, so these contributions can be ignored. In estimating the meridional temperature gradient, I have also omitted effects of compressibility and used *in situ* temperature. Depth-integrated meridional heat flux, given by the product of K_y and the meridional temperature gradient, is shown in Fig. 1d.

Figure 2 shows total eddy heat transport, integrated across the width of the Pacific, as a function of latitude. The result has a bearing on a controversy concerning North Pacific heat transport. Geostrophic section calculations¹² at 35° N indicate a southward mean transport of $(0.1-0.5) \times 10^{15}$ W. Detailed studies of exchanges of radiant, latent and sensible heat at the sea surface have produced conflicting results, suggesting¹³ that total oceanic heat transport across 35° N is small and of indeterminate sign. Adding the eddy transport from Fig. 2 to the geostrophic section estimates yields a sum that is small and of indeterminate sign.

Finally, the calculations given above for eddy heat transport have been repeated for eddy salt transport, based on the 3,000-m depth-averaged salinity field. Figure 2 shows eddy salt transport, integrated across the width of the Pacific. Mean salt transports from geostrophic section calculations have not been reported, so it is not possible to estimate the total salt transport. However, we may inspect the eddy salt transport for gross inconsistency with the North Pacific fresh water budget, as this would decrease confidence in the eddy heat transport calculation. Although precipitation and runoff are believed to exceed evaporation north of 35° N, there is considerable uncertainty, such that estimates (refs 14, 15 and G. McBean, personal communication) of net fresh-water supply range from near zero to 3.5×10^8 kg s⁻¹. Eddy salt transport of 5×10^6 kg s⁻¹ at 35° N is thus consistent with observed salinity, given the wide uncertainty in net fresh-water supply. This consistency test will need to be reconsidered if and when estimated mean salt transports from geostrophic sections are reported.

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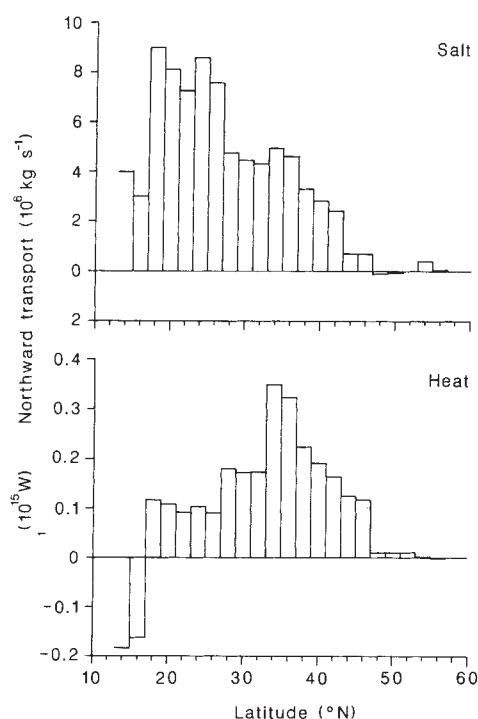


Fig. 2 Northward eddy transports of heat and salt, integrated across the longitudinal extent of the Pacific, and plotted as functions of latitude.

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Empirical relation between sulphur dioxide emissions and acid deposition derived from monthly data

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The relation between sources of sulphur dioxide (SO_2) and precipitation sulphate (SO_4) concentrations at distant receptors is the subject of intensive empirical and theoretical investigation^{1,2}. This relation provides a probe of atmospheric chemistry, physics and meteorology, and insight into the effectiveness of potential acid-deposition reduction strategies. Large variations in SO_2 emissions from copper smelters in the southwestern United States, the major regional sulphur source, between 1980 and 1984 provide a unique opportunity to study the source-receptor relation, as during this interval the National Atmospheric Deposition Program (NADP) monitored wet-precipitation sulphate concentrations on a weekly basis at Rocky Mountain locations. Here we extend our earlier analysis of annual-average data² by examining monthly sulphate concentration and emission data for 1980–84. We show that monthly data are consistent with a linear relation between emissions and concentration, possessing the expected properties of a source-receptor relation. We then predict concentration changes resulting from the addition of a new smelter at Nacozari, Mexico, expected to be the second-largest source of SO_2 in North America.

Sulphur dioxide emissions from 11 copper smelters in Arizona, New Mexico, Utah and Nevada were summed to yield monthly regional emissions by the smelter source sector (Table 1, Fig. 1). The uncertainties in the emissions inventory are estimated at about $\pm 5\%$ (ref. 2). Ionic concentrations in wet precipitation have been measured weekly at five Colorado monitoring stations (Sand Spring, Pawnee, Rocky Mountain National Park, Alamosa and Manitou; see ref. 2, Fig. 1) since mid-1980³. In the NADP data, the error due to imprecision in sampling and analysis is $<10\%$ (ref. 4).

Weekly observations from the five Colorado stations were combined and averaged over each month to produce volume-weighted mean (VWM) concentrations, shown in Table 1 and Fig. 1. Valid samples were available for $>80\%$ of the station-weeks between June 1980 and December 1984. Monthly averages from the combined weekly records of five stations are studied because they are subject to a smaller standard error than are monthly station-specific averages. We use volume-weighted averages in order to reduce the effect of low-volume events, for which concentration is volume-dependent⁵. We have restricted ourselves to this set of the five longest-recording stations in a single state because the use of data from more widely separated stations adds an element of geographical variability, increasing the standard error of the VWM concentrations.

To investigate the relation between emissions and sulphate concentration, we use a linear regression model. A regression of 55 consecutive months of VWM sulphate concentration data against total smelter SO_2 emissions explains little of the variation in sulphate and is marginally insignificant (see Table 2, line 1). However, 13 of the 55 months fail to meet the NADP quality-control criterion that chemical data be available from at least 75% of the station-weeks in an averaging period. If these 13 months are omitted from the data set, then regression of VWM sulphate against smelter SO_2 explains 18% of the concentration variability ($P < 0.01$, *t*-test; see Table 2, line 2).

By inspection, Fig. 1 reveals annual periodicity in VWM sulphate concentration variability, with higher concentrations in the spring and summer months. As this periodic variation is due to seasonal changes in regional photochemistry and

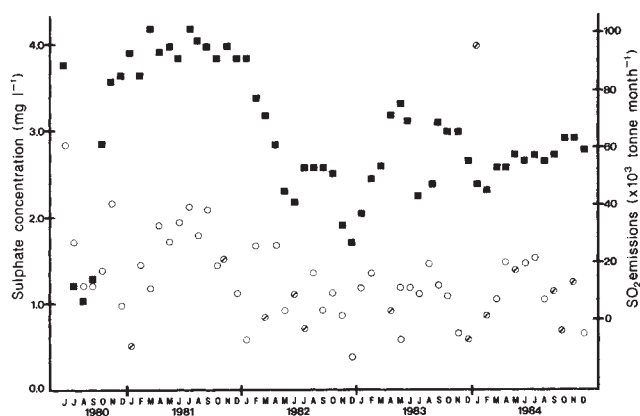


Fig. 1 Smelter SO_2 emissions and VWM sulphate concentrations from five Colorado stations, combined, for June 1980 to December 1984. ■, Emissions; ○, concentration; Ø, incomplete concentration data (that is, chemical data available from $<75\%$ of station-weeks in the month).

meteorology, and not to periodic variation in smelter emissions, we have used a computer algorithm⁶ to remove the annually periodic component of VWM sulphate concentration variability. The algorithm partitions a monthly time series into trend, seasonal and irregular components, using a series of moving median, moving average, and weighted moving least-squares-regression filters. The seasonal component is allowed to evolve over time. The de-seasonalized series (Table 1) is the sum of the trend and irregular components, and is calculated from the entire 55-month series.

Regression of quality-controlled de-seasonalized sulphate on smelter emissions explains 41% of the variation in the sulphate series ($P < 0.001$, *t*-test; see Table 2, where regression of non-quality-controlled de-seasonalized data is also displayed). Addition of a quadratic term to the regression model did not significantly increase the proportion of variance explained in de-seasonalized sulphate, indicating that the SO_2 – SO_4 relation is insignificantly nonlinear. Figure 2 shows the linear regression of de-seasonalized, quality-controlled sulphate against smelter emissions. Concentration residuals from the regression of de-seasonalized concentration against emissions lack significant first-order autocorrelation, whether ordered as a time series or by emissions.

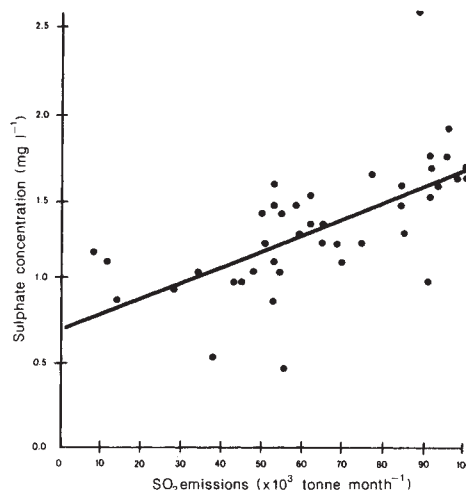


Fig. 2 De-seasonalized VWM monthly sulphate concentrations for five Colorado stations, combined, plotted against total smelter SO_2 emissions in the inter-mountain region for June 1980 to December 1984, using quality-controlled data set ($n = 42$). Also shown is the regression line between the two data sets.

We now consider whether the statistical relation between Colorado precipitation sulphate and smelter SO_2 possesses the expected physical properties of a true source-receptor relation. Sulphate concentration, C , may be expressed as a function of E_s and E_b , smelter and background emission, respectively: $C = k_s E_s + k_b E_b$, where k_s and k_b are proportionality constants. From Fig. 2, the x -intercept (E_s at $C = 0$, or $-(k_b/k_s)E_b$) is $-71,000$ (metric) tonnes (95% confidence limits (CL): $-33,000$, $-157,000$), calculated as the y -intercept divided by the slope. E_b is estimated at 33,000 tonnes per month for inter-mountain United States sources⁷⁻⁹. Thus, the slope and intercept of the line in Fig. 2 are consistent with an independent estimate of background emissions, as the relative distance of smelter and background sources from Colorado receptors leads us to expect that $k_b > k_s$. E_b is treated as a constant here because background emissions are dominated by electric power plant emissions, which deviate from their annual mean values during any given month by no more than 10% of total regional emissions, and are constant to within a few per cent from year to year⁹. Natural sulphur sources such as airborne soil sulphate may also affect the intercept.

The slope and intercept of the regression line in Fig. 2 imply that in 1981, the period of highest smelter emissions, the contribution of average smelter emissions to sulphate concentrations was 57% (95% CL: 38%, 74%). By comparison, a theoretical long-range transport model based on regional meteorology estimated up to a 46% smelter contribution to Colorado precipitation sulphate in 1981¹⁰. Furthermore, a regional mass budget study concluded that most wet sulphate deposited in Colorado in 1981 originated in out-of-state emissions¹¹.

The Arizona smelters are the largest single-state source of SO_2 , whether compared with smelters in New Mexico, Utah and Nevada, or with electric utility emissions (inventory available through 1983) from Colorado, Utah, Arizona, New Mexico, Nevada and Wyoming^{9,12}. Consistent with this distribution of source magnitudes, when de-seasonalized sulphate is multiply regressed against the 10 source categories (4 smelter states plus 6 electric utility states), only Arizona smelter SO_2 explains a significant portion of the variation in sulphate ($n = 36$ months for which quality-controlled NADP data are available between June 1980 and December 1983: $P < 0.01$, F -test).

Three additional points of physical interpretation deserve mention. First, photochemical processes are slower in the winter and upper-level wind trajectories arise less often from the south and west in winter than in summer, so one expects a weaker relation in winter. In fact, we find significant relations for the second, third and fourth quarters of the year but not for the first quarter. This difference may also reflect the poorer data quality in that quarter. Second, a decreasing regression coefficient (slope) with increasing distance from smelter sources has been demonstrated when all NADP stations in the region are examined¹³. Thus, the relation possesses the expected geographical structure. A transport scale length for sulphur may be derived from the concentration gradients or slope gradients: this value is nearly 1,000 km (refs 2, 13). Third, in addition to sulphate, magnesium concentrations (but no other ion concentrations) are related to SO_2 emissions. This relation may reflect SO_2 control of soil dust solubility in droplets.

Considerable variance remains in monthly sulphate, unexplained by smelter emissions. Meteorological fluctuations in wind trajectories and precipitation amounts influence precipitation concentrations. Other sources of variation in VWM sulphate include changes in background emissions, lagged responses due to sulphur transport times, and shifts in the geographical balance represented by the VWM concentration due to changes in monthly precipitation volume at each station.

A test of this source-receptor relation will be provided shortly, as a copper smelter at Nacozari, Mexico, 55 miles south of the Douglas, Arizona smelter has recently started to operate. If uncontrolled, this smelter will emit 500,000 tonnes of SO_2 per

Table 1 Smelter SO_2 emissions and volume-weighted mean sulphate concentration from five Colorado stations, combined

Date	Total emissions (ktonne month ⁻¹)	Concentration (mg l ⁻¹)	De-seasonalized concentration (mg l ⁻¹)
1980			
June	88.35	2.86	2.55
July	11.02	1.69	1.09
August	6.83	1.17	1.15
September	13.43	1.18	0.86
October	61.76	1.39	1.29
November	83.74	2.18	1.53
December	84.03	0.94	1.43
1981			
January	92.05	0.52*	1.97
February	84.50	1.43	1.27
March	101.14	1.15	1.59
April	92.94	1.88	1.50
May	95.75	1.67	1.68
June	90.98	1.94	1.71
July	101.10	2.12	1.67
August	97.92	1.77	1.62
September	95.68	2.09	1.88
October	91.24	1.45	1.45
November	95.99	1.59*	1.35
December	91.05	1.10	1.62
1982			
January	91.28	0.56	0.99
February	77.05	1.68	1.61
March	70.64	0.80*	1.25
April	61.69	1.70	1.47
May	44.36	0.92	0.95
June	41.64	1.07*	0.92
July	52.31	0.67*	0.37
August	52.13	1.36	1.07
September	52.06	0.93	0.83
October	50.32	1.08	1.19
November	33.79	0.86	1.04
December	27.32	0.38	0.94
1983			
January	37.53	1.14	0.53
February	49.53	1.33	1.37
March	53.80	0.56	1.03
April	71.54	0.90*	0.81
May	74.58	1.15	1.20
June	69.43	1.15	1.08
July	43.26	1.09	0.94
August	47.53	1.46	1.04
September	68.13	1.18	1.19
October	64.32	1.06	1.28
November	64.51	0.63	1.22
December	55.62	0.56*	1.15
1984			
January	49.54	3.88*	2.21
February	47.06	0.83*	0.98
March	53.66	1.03	1.51
April	52.89	1.38	1.43
May	58.14	1.28*	1.35
June	55.34	1.38	1.39
July	54.39	1.43	1.42
August	51.85	1.01	0.46
September	53.51	1.07*	1.20
October	63.32	0.65*	0.98
November	63.94	1.20*	2.20
December	59.90	0.62	1.24

Arizona emission values are from the Arizona Department of Health Services, Phoenix. Emission values for all non-Arizona Kennecott sources (in Utah, Nevada and New Mexico) are from the Kennecott Minerals Company, Salt Lake City, Utah. Due to malfunctioning monitors at the Hurley, New Mexico smelter during 1980 and the last quarter of 1984, emissions for these months are estimated from available monthly data and a knowledge of operating days per month at this site. Annual and quarterly emissions for the Phelps Dodge smelter in Hidalgo, New Mexico were obtained from the Air Quality Bureau, New Mexico Department of Health and Environment, Santa Fe. Monthly emissions were estimated as one-third of quarterly during the corresponding quarter, except for late 1980, when they were estimated as one-twelfth of annual. Monthly sulphate concentrations were calculated as the volume-weighted mean of weekly observations, combined from five Colorado stations of the NADP (Sand Spring, Pawnee, Rocky Mountain National Park, Alamosa, Manitou).

* Concentration from month failing to meet the NADP quality-control criterion that chemical data be available from at least 75% of the station-weeks in the averaging period.

Table 2 Statistical properties of regressions of sulphate against total smelter SO₂ emissions

Dependent variable	n (months)	Explained sum of squares as % of total	P <	Slope (95% CL) (mg l ⁻¹ ktonne ⁻¹ month ⁻¹)	y-intercept (95% CL) (mg l ⁻¹)
Monthly VWM [SO ₄]	55	6	0.100	0.0064 (-0.0005, 0.0133)	0.87 (0.40, 1.33)
Monthly VWM [SO ₄]	42	18	0.010	0.0083 (0.0026, 0.0140)	0.78 (0.39, 1.17)
De-seasonalized [SO ₄]	55	28	0.001	0.0094 (0.0053, 0.0135)	0.69 (0.41, 0.97)
De-seasonalized [SO ₄]	42	41	0.001	0.0094 (0.0058, 0.0130)	0.69 (0.44, 0.94)

Regressions based on 42 points have omitted months with incomplete concentration data. The last two regressions are based on seasonally adjusted data.

yr at full operation. From Fig. 2, we predict an increase in long-term monthly average precipitation sulphate concentrations at these five Colorado stations to nearly 1.60 mg l⁻¹ (95% CL: 1.45, 1.75) if background emissions, meteorology and oxidant levels resemble those for the 1980–84 period, and domestic smelters emit at their 1984 level. An indistinguishable prediction is obtained if the regression is based on Arizona smelter emissions only. This predicted concentration approaches the highest average values observed over the period. Observation of the response of the atmosphere in this inadvertent experiment

will provide a unique opportunity for studies of regional atmospheric chemistry and transport. It is imperative that coordinated deposition, cloud chemistry, aerosol, gaseous concentration, tracer and surface water chemistry measurements be planned around this source modulation as the smelter increases production in the next few years.

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Late-glacial climatic oscillation in Atlantic Canada equivalent to the Allerød/younger Dryas event

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Attempts^{1–5} to relate late-glacial events in northeastern North America to the well-documented climato- and chrono-stratigraphy of Europe and the British Isles⁶ have not been considered convincing because the evidence presented was from isolated sites, and could therefore be interpreted as local fluctuations not related to a general, widespread climatic change^{7–10}. However, recent palynological studies in northeastern North America postulating a late-glacial climatic oscillation^{11–15} have caused renewed interest in relating such an oscillation to the Allerød/younger Dryas event. We have extended our preliminary results from Nova Scotia and New Brunswick^{13,14}, and here present the evidence for a climatic event in Atlantic Canada. This event involved a warming trend before 11,000 yr BP that was interrupted by a cold period which persisted until the abrupt Holocene warming at ~10,000 yr BP. We propose a possible mechanism to link this event with that of Europe.

The event is recorded in buried organic deposits and lake sediments. Buried organic deposits are shallow pond and mire sediments and organic soils that began to accumulate sometime after deglaciation, as vegetation invaded the region. These

organic sediments were subsequently buried by mineral sediments which were deposited in response to a widespread and synchronous climatic disturbance of sufficient magnitude to strongly affect depositional environments throughout Atlantic Canada, and are now exposed in sections along coasts and river banks and in excavations (pits and quarries).

Fourteen buried organic sites dating from late-glacial time have been studied (Fig. 1). Dates from narrow increments (≤2 cm) at the top and base of the organic horizon, or from thin organic seams, were obtained from eight sites; other dates are for bulk organic matter or for only one level within the organic horizon (Fig. 2; Table 1). Typical profiles illustrating palynological changes at three widespread localities are shown in Fig. 3a. Only the major taxa are plotted; less abundant taxa are grouped as other trees, shrubs and herbs.

At Lantz, basal organic sediments dated at 11,700 yr BP contain abundant sedge (*Cyperaceae*) and fern (*Pteridophyta*) palynomorphs, along with birch (*Betula*), grass (*Gramineae*) and a variety of other shrubs and herbs. Increased spruce (*Picea*) pollen and the presence of spruce needles within the organic unit show that spruce trees had migrated into the area. The top of the peat is dated at 10,900 yr BP. In the overlying mineral sediments, sedge, wormwood (*Artemisia*) and birch (shrub type) become dominant and spruce declines.

The Campbell sequence begins just before 11,300 yr BP. A succession is observed from sedge-dominated spectra through peaks in willow (*Salix*), rose (mainly *Spiraea*) (*Rosaceae*) and birch (shrub type), indicating a change from herbaceous to shrub tundra. Spruce trees were not present, although a small spruce peak following the birch maximum may indicate that spruce was approaching the area. A reverse trend to *Spiraea*, willow and sedge follows, preceding the change from organic to mineral sedimentation. Pollen influx rates increase considerably with the incursion of shrub taxa and decline abruptly when they are displaced.

The profile at Benacadie Point, dated at ~12,100 yr BP at the base, begins with abundant willow, grass and sedge pollen,

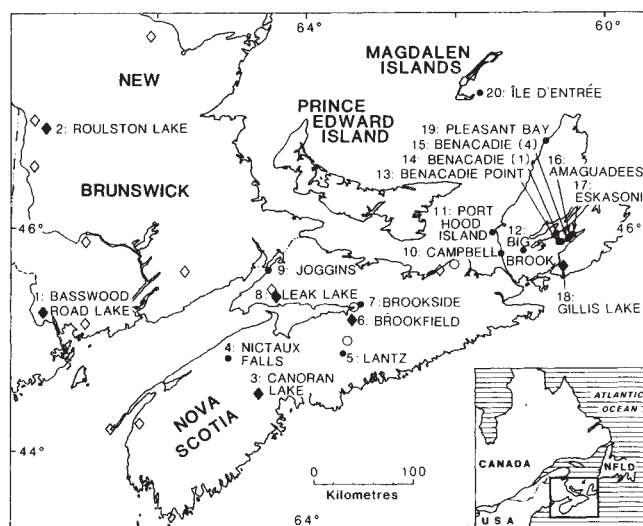


Fig. 1 Location map showing: ●, buried organic sites; ◆, lake sites; ○, buried organic sites where palynology and chronology are not yet available; ◇, lake sites with lithological changes similar to those at sites described here, but where palynology and chronology are not yet available.

indicative of shrub tundra. Grass pollen declines slightly as birch (shrub type) and sedge increase. Near the top of the organic sediments, dated at 10,900 yr BP, willow increases slightly again. Pollen influx rates are slightly higher in the upper organics but are ≤ 100 grains $\text{cm}^{-2} \text{yr}^{-1}$, indicating depauperate tundra.

Six lake sites (Fig. 1) document vegetational and climatic changes from 12,600 yr BP. Lithology and dates (Fig. 2) show that early organic accumulation was replaced at $\sim 11,000$ yr BP by mineral sedimentation, which was followed by a return to organic sedimentation after 10,000 yr BP. The pollen profiles record the same vegetational changes, indicative of an early warming trend, as in the buried organic deposits, and also a subsequent climatic deterioration and a prominent early Holocene warming. Three lake-sediment profiles illustrate these changes (Fig. 3b).

The Basswood Road Lake diagram¹⁶ shows shrub tundra spectra below the 12,600 yr BP date, followed by successive peaks in poplar/aspen (*Populus*) and spruce pollen up to the clay contact. Pollen influx values suggest progression from tundra to boreal woodlands. Organic content steadily increases. These trends are interrupted above the 11,300 yr BP date by more minerogenic sediments, a decline in spruce and increases in shrub and herb pollen, suggesting a more open vegetation cover and less stable slopes. A reversal to organic sediments and more tree and less shrub and herb pollen follows.

At Roulston Lake, shrub-birch-dominated tundra was replaced after 11,100 yr BP by herb tundra dominated by *Artemisia* and sedges. Trees, including birch, poplar/aspen and spruce, arrived after $\sim 10,000$ yr BP and shrubs and herbs declined. Organic content declined until after 10,000 yr BP.

The Gillis Lake diagram¹ shows early shrub/herb/tundra spectra displaced by abundant birch (probably shrub birch) pollen as sediment organic content increases. Birch pollen then declines and sedge becomes abundant, coincident with a decline in sediment organic content. The Holocene increase in vegetation is reflected above the 10,160 yr BP date¹⁷ by increasing birch and spruce and declining shrub and herb pollen, and by organic sediments.

Thus, the lithology, palynology and chronology indicate increasing organic sedimentation and proliferation of vegetation by primary succession in response to a stable or slightly warming climate before 11,000 yr BP. The climatic threshold for trees had been attained, or the climate was gradually warming to allow vegetation to immigrate by normal succession. Slopes stabilized and organic sedimentation predominated in basins. Spruce and poplar/aspen trees had invaded southern New Brunswick and spruce had reached southwestern Nova Scotia to form open woodlands. Shrub and herb tundra prevailed farther north in

New Brunswick and in northeastern Nova Scotia. At $\sim 11,000$ yr BP, vegetation reverted to fewer trees and more shrubs and herbs in the south, and fewer shrubs and herbs in the north and north-east. Thinning vegetation destabilized soils, causing mineral sediments to bury shallow pond sediments, peatlands and soil profiles; mineral sedimentation dominated in lakes. Increased frost action, solifluction and mass movements may be implicated at some sites, and increased river bed-loads and aeolian activity at others. Increased rainfall or snowfall may have been a factor. The overlying till-like diamictos suggest the possibility of glacial activity, but re-activation or reglaciation has not been demonstrated. However, in Newfoundland¹⁸ and

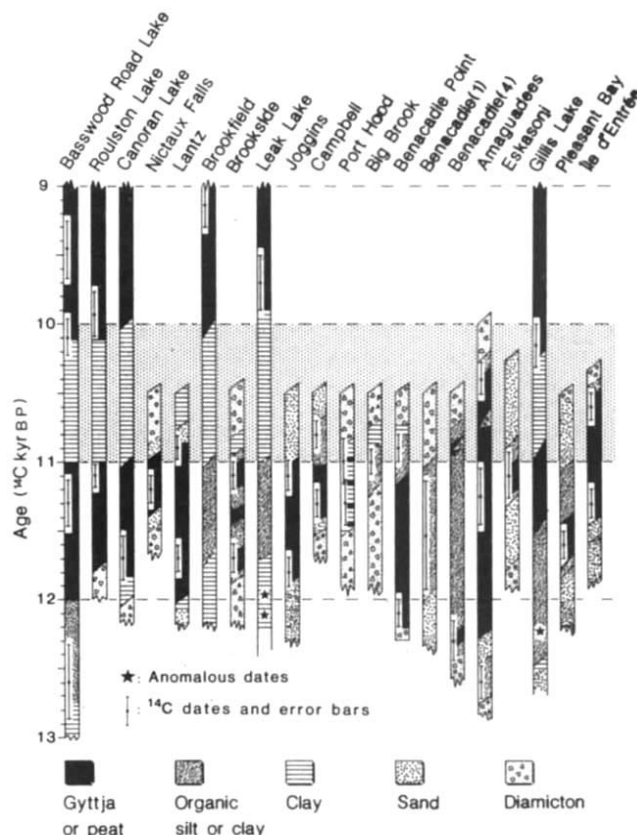


Fig. 2 Schematic diagram showing the stratigraphy and radiocarbon dates for 14 buried organic and 6 lake sediment sites. Stippled pattern denotes period of climatic cooling. Anomalous dates are listed in Table 1. TC, Total carbon; LOI, loss of ignition.

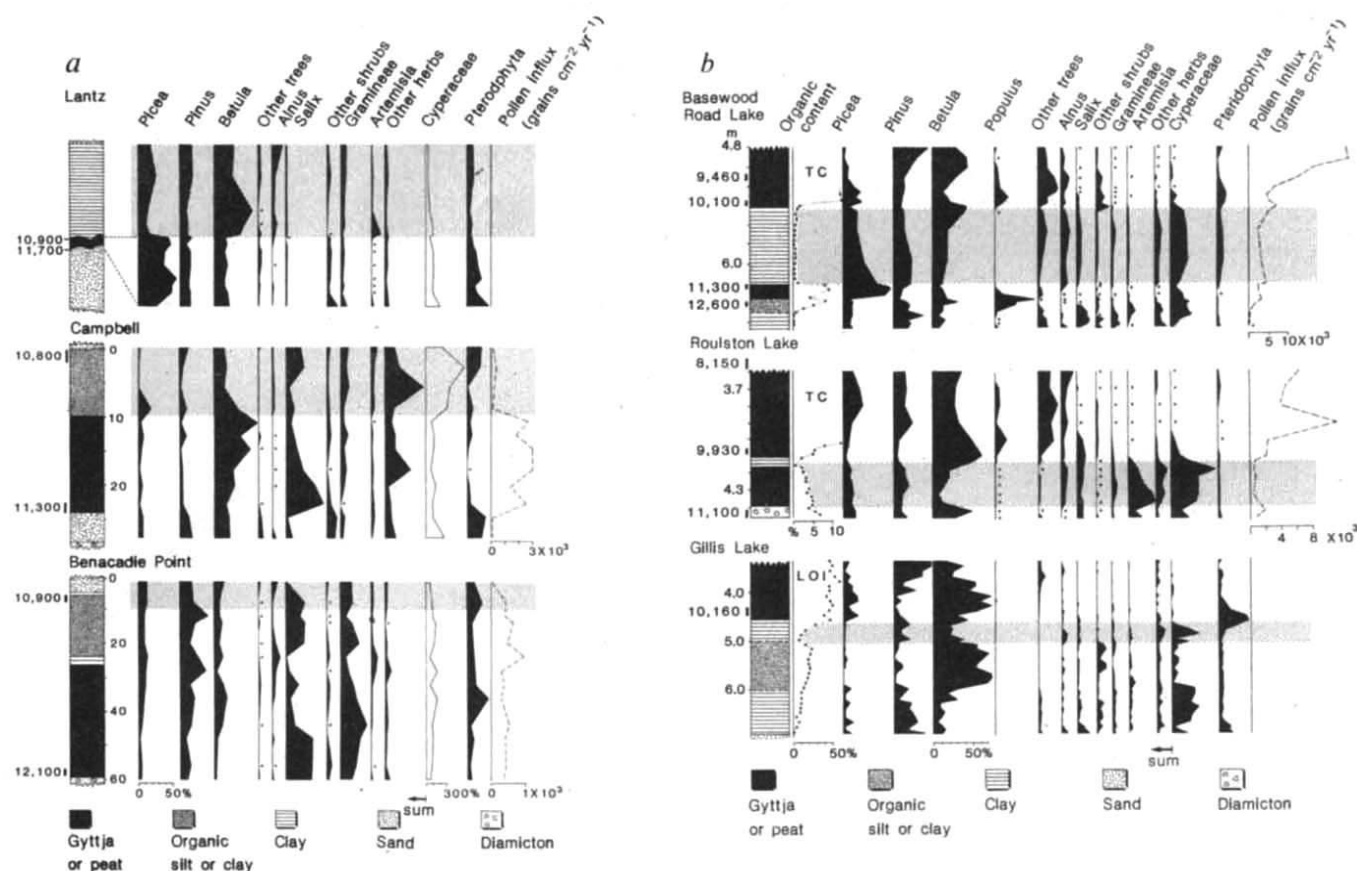


Fig. 3 Pollen diagrams for three buried organic sites (*a*) and three lake sediment sites (*b*), showing only the major taxa. Less abundant taxa are grouped as 'Other trees', 'Other shrubs' and 'Other herbs'. Stippled pattern denotes period of climatic cooling. The Gillis Lake diagram is adapted from ref. 1.

southern Quebec¹⁹, re-advance of remnant glaciers has been documented. All evidence points to a distinct and abrupt climatic deterioration. This cool episode continued until almost 10,000 yr BP, when organic deposition again became dominant and vegetation proliferated in response to a major climatic amelioration.

The chronology of late-glacial climatic change is well documented in Europe, particularly northwestern Europe and the British Isles. There is general agreement on the chronology⁶, despite the usual problems in precisely dating vegetational and inferred climatic changes^{20,21}.

The record of late-glacial climatic change in Atlantic Canada is broadly parallel to that of Europe. The earliest dates for non-marine organic sedimentation are ~12,600 yr BP or slightly earlier, depending on the validity of dates from Newfoundland^{12,15}. Vegetational changes correlative with the Bølling and Allerød followed a pioneering succession from herbs and shrubs to the invasion of trees. The older Dryas cool period is apparently not represented. A reversion to cooler climate began near 11,000 yr BP, judging by the many dates from buried organic deposits. This cool period lasted until 10,000 yr BP, although slight climatic amelioration before this date is seen in the timing of the return to organic sedimentation at some lake sites (for example, Basswood Road Lake and Gillis Lake). The abrupt and major warming apparent in the pollen record throughout northeastern North America, particularly in the influx record, occurred at 10,000 yr BP or shortly thereafter²².

The correspondence of deglacial climatic evolution in Europe and North America may be linked to oceanic conditions. Studies of North Atlantic deep-sea cores²³⁻²⁶ have shown that the deglacial warming that began ~13,500 yr BP was reflected as a gradual retreat of the Arctic polar front from its near-glacial east-west position at ~40° N to a position near Greenland and Iceland at ~9,200 yr BP. This warming trend was interrupted

when the polar front moved southeastwards again to near its full glacial position, between ~11,000 and 10,000 yr BP²⁶. Recent work on an ash bed in Norway, dated at ~10,600 yr BP²⁷, which may correlate with the North Atlantic ash bed used for stratigraphy and chronology, suggests that the return of the polar front to near its full glacial position may have occurred earlier. The movement of the polar front thus affected northwestern Europe, to cause the Allerød warming and younger Dryas cooling. The supposed stability of the polar front in the western North Atlantic has been cited to account for the apparent lack of an equivalent synchronous climatic oscillation in northeastern North America^{6,9}. Our findings suggest that there was a comparable shift in the western North Atlantic; thus, we postulate a single mechanism to account for the cooling episode on both continents. The age of the onset of climatic cooling in the North Atlantic, as suggested by the age of the Norway ash bed²⁷, would accord with the beginning of the climatic deterioration registered in Atlantic Canada.

Another possible mechanism concerns the air-temperature anomaly that would result from surface ocean temperatures north of the polar front during the younger Dryas. Computer modelling suggests that cooler air temperatures would be generated in Atlantic Canada as well as in northwestern Europe²⁸.

The younger Dryas cooling may not be simply a North Atlantic regional phenomenon resulting from ice shelf break-up⁸, melt-water diversion²⁹, reorientation of atmospheric flow³⁰, or deep-water production²⁸. Recent suggestions that the younger Dryas cooling equivalent may be recorded farther inland in continental North America^{11,31}, and elsewhere^{28,32}, suggest that the underlying cause or causes may be more complex than has been thought, and global cooling may be implicated³³.

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Table 1 Radiocarbon dates for buried organic and lake deposits

Site	Increment dated (cm)	Lab. no.	Age (^{14}C yr) before 1950	^{13}C (‰)	Ref.
1. Basswood Road Lake	507-512	GSC-1643	9,460 $\pm$ 220	-27.5	16
	535-540	GSC-3862	10,100 $\pm$ 130	-26.4	
	621-626	GSC-1645	11,300 $\pm$ 180	-27.4	
	639-644	GSC-1067	12,600 $\pm$ 270		
2. Roulston Lake	348-358	GSC-3455	8,150 $\pm$ 130	-32.8	16
	406-409.5	GSC-2872	9,930 $\pm$ 160	-24.8	
	440-446	GSC-2804	11,100 $\pm$ 90	-23.2	
	345-355	GSC-1486	11,700 $\pm$ 160		
3. Canoran Lake	Bulk	GSC-2062	11,200 $\pm$ 100		34
4. Nictaux Falls	Top 1 cm	GSC-3771	10,900 $\pm$ 90	-28.4	36
5. Lantz	Bulk	GSC-3116	11,100 $\pm$ 100		
	Basal 1 cm	GSC-3774	11,700 $\pm$ 100	-30.5	
6. Brookfield	96.5-98	GSC-3635	9,140 $\pm$ 170		
7. Brookside	190-196	GSC-2930	11,100 $\pm$ 100		37
	242-250	GSC-3849	11,700 $\pm$ 110		
8. Leak Lake	329-341	DAL-314	9,715 $\pm$ 200		38
	433-438	GSC-2728	12,900 $\pm$ 160*	-30.2	38
	445-450	GSC-2880	15,900 $\pm$ 1200*	-28.3	38
	Top 2 cm	GSC-3924	11,100 $\pm$ 120	-25.1	
9. Joggins	Bulk	GSC-3550	11,400 $\pm$ 100		37
	Basal 2 cm	GSC-3915	11,800 $\pm$ 110	-26.4	
10. Campbell	Top 2 cm	GSC-3892	10,800 $\pm$ 100	-23.4	
	Bulk	GSC-2212	11,200 $\pm$ 110		
11. Port Hood Island	Basal 2.5 cm	GSC-3781	11,300 $\pm$ 100	-28.5	39
	4-9	GSC-540	11,000 $\pm$ 170		
12. Big Brook	49-56	GSC-541	11,300 $\pm$ 160		40
	Bulk	GSC-3378	11,000 $\pm$ 90	-27.5	
13. Benacadie Point	Top 2 cm	GSC-3912	10,900 $\pm$ 100	-27.0	40
	Bulk	GSC-2146	11,300 $\pm$ 90		
	Bulk	I-3234	11,670 $\pm$ 170		
	Basal 2 cm	GSC-3900	12,100 $\pm$ 100	-27.5	
14. Benacadie (1)	Bulk	UQ-395	11,530 $\pm$ 390		41
15. Benacadie (4)	Bulk	UQ-394	12,310 $\pm$ 150		
16. Amaguadees	Top 2 cm	GSC-4063	10,400 $\pm$ 120	-27.4	
	Bulk	UQ-970	11,250 $\pm$ 200		
17. Eskasoni	Basal 4 cm	GSC-4062	12,600 $\pm$ 120	-27.5	
	Bulk	UQ-831	11,100 $\pm$ 170		
18. Gillis Lake	431-456	Y-524	10,160 $\pm$ 160		17, 1
	550-590	Y-525	13,450 $\pm$ 260*		
19. Pleasant Bay	Bulk	UQ-971	11,600 $\pm$ 100		
20. Île d'Entrée	Top 1 cm	GSC-3699	10,600 $\pm$ 100	-27.9	
	Bulk	GSC-3657	11,100 $\pm$ 70		
	Basal 2 cm	GSC-3696	11,300 $\pm$ 110	-27.2	

* Dates considered to be anomalously old.

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In situ studies of megafaunal mounds indicate rapid sediment turnover and community response at the deep-sea floor

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Sediment mounds, composed of faecal material from megafaunal deposit feeders^{1,2}, occur in virtually all deep-sea environments, forming the dominant decimetre-scale topography on the ocean floor^{3,4}. Mounds are often zones of deep, convective bioturbation^{1,2}, and their formation is thought to disturb infaunal benthos^{5,6}. We are studying mounds in the deep sea to evaluate rates and patterns of megafaunal sediment mixing, and to assess the disturbance effects of mound building on the infaunal community, with the goal of using mounds to investigate natural processes of succession at the deep-sea floor⁷. Our results indicate that sediment turnover associated with mound building by echiuran worms can be very rapid, and that bathyal infauna can respond quickly to this type of disturbance. These findings suggest that spatially rare but dynamic elements in the sediment fabric, such as mounds, may be important in controlling microstratigraphy, chemical diagenesis and community structure at the deep-sea floor.

At our study site at a depth of 1,240 m in the Santa Catalina Basin⁸ (33°12' N, 118°30' W), large mounds (~10 cm high and 30 cm in diameter) are relatively abundant, covering ~2% of the sea bed. The most common type of mound is apparently formed of faecal pellets from a large echiuran worm, probably *Prometor benthophila*⁹. The echiuran appears to feed on surface sediments in a shallow pit (~10 cm deep and ~1 m diameter)¹⁰, depositing its faeces by means of U-shaped burrows on one of several peripheral mounds⁹⁻¹².

Several lines of evidence indicate faecal deposition rates on mounds of ~1–2 cm per month, that is, rates ~1,000 times higher than those of long-term sediment accumulation in the Santa Catalina Basin (SCB)¹³. The most dramatic evidence comes from time-lapse, stereo photographs. These were obtained *in situ* from a camera tripod placed by the research submersible *Alvin* over a mound from 17 December 1984 to 4 February 1985. Photographs taken at 10-h intervals yielded sequential profiles (Fig. 1) which indicate ~1–2 cm of deposition out to a radius of 5–8 cm from the central burrow during the 120 h immediately following camera deployment. This growth episode was followed by a 30-day quiescent period, during which no appreciable deposition took place, even though the echiuran was photographed in its burrow.

Depth distributions of tracer beads spread on the surface of mounds also indicate faecal deposition rates of 1–2 cm per month. *Alvin* spread spherical glass beads (5–420 µm diameter, 2.42 g cm⁻³ specific gravity) on three steep-sided (and therefore active) mounds, and in three control areas, between 14 and 16 December 1984; the submersible returned and collected core samples on these marked sites ~50 days later. Cores were analysed for beads at 1-cm depth intervals. All three marked mounds exhibited tracer beads to depths of 1–3 cm, with peak bead abundance typically occurring in the 1–2-cm layer; in control sites, beads were essentially restricted to the top centimetre of sediment (Fig. 2). Sub-surface peaks in tracer distributions on mounds suggest that sediment deposition, rather than diffusive sediment mixing, 'moved' beads downward¹⁴, indicating mound growth rates of ≥1 cm per month. In addition, two mounds showed marked secondary bead peaks at depths of 6–8 cm (Fig. 2); this suggests transport of surface sediments

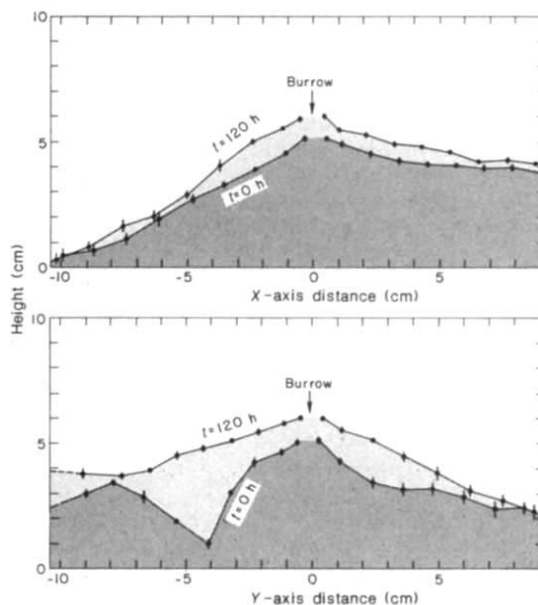


Fig. 1 Cross-sectional profiles through the peak of an echiuran mound, drawn from stereo photographs using a stereocomparator. Sets of profiles are drawn along two normal axes, from photographs taken shortly after the camera was emplaced ($t = 0$ h) and 120 h later ($t = 120$ h). Each point represents the mean ($\pm$ s.e.) of five replicate runs with the stereocomparator. Heights are referenced to an arbitrary base level.

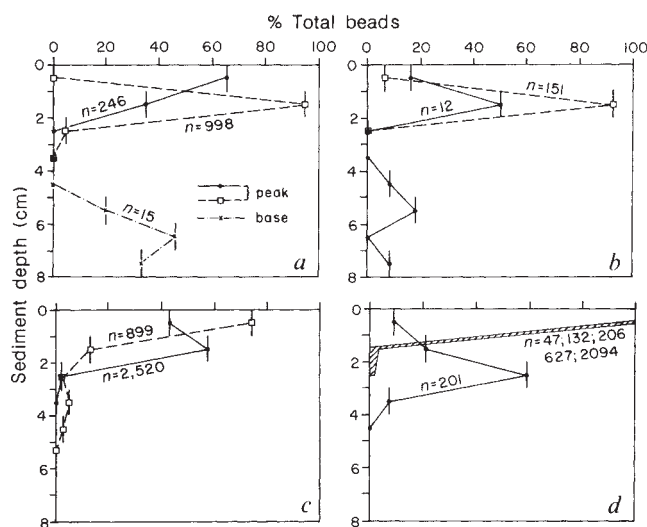


Fig. 2 Vertical distributions in the sediment of glass beads (208–420 µm diameter, that is, the approximate *in situ* grain size of pelletized SCB sediment) spread at the sediment-water interface 50 days before sampling. The number of beads in each profile is indicated (n). a, Vertical profiles near the peak and at the base of a mound (1510-E2); b, c, profiles near the peaks of mounds (1512-A5 and 1512-A6, respectively); d, profiles from background sediments (control sites). The hatched area in d encloses curves from five control cores. The sub-surface peak in one of the control profiles apparently results from bead penetration of an animal burrow.

Methods. Beads were spread from *Alvin* using a screen-covered dispenser. Core samples (~30 cm long) were taken 50 days later in labelled areas with 400-cm² Ekman corers or 35-cm² *Alvin* corers⁶. On board ship, these cores were subsampled for beads, using 2.2-cm-diameter syringe corers inserted to a depth of 9 cm. Syringe cores were extruded, cut off at intervals of 1 cm and preserved in formalin. Beads retained on a 208-µm sieve were subsequently counted using dark-field microscopy. Data from the 8–9-cm depth intervals of bead cores are not presented because core-tip entrainment of beads caused contamination at this level.

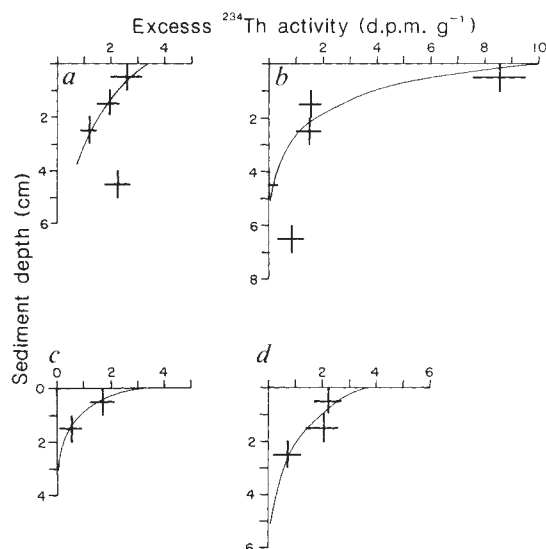


Fig. 3 Depth distributions of excess ^{234}Th activity in four active mounds. Measurements (crosses) were made over vertical intervals of 1 cm; horizontal error bars indicate ± 1 s.d. from counting statistics. Curves indicate expected distributions of ^{234}Th excess given constant surface activity and deposition rates (w), and assuming minimal sediment mixing¹⁶. a, Mound 1491, $w = 2.2$ cm per month; b, mound 1499, $w = 1.0$; c, mound 1500-1, $w = 0.7$; d, mound 1500-2, $w = 1.4$.

Methods. ^{234}Th measurements were made on 35-cm² core samples collected from mounds using *Alvin*. On board ship, cores were extruded and sectioned at 1-cm intervals; the outside 'rind' (~2 mm) of each sampled interval was discarded to avoid contamination resulting from core insertion. Samples were counted using the methods of ref. 16.

down the echiuran burrow, and indicates a minimum 8-cm mixing depth associated with mounds.

Depth distributions of excess ^{234}Th on four mounds also suggest rapid, episodic deposition. ^{234}Th , a decay product of ^{238}U with a 24-day half-life, is produced in the water column and scavenged by sinking particles¹⁵. The excess ^{234}Th signal picked up by particles during transit through the water column remains detectable in bottom sediments for ~100 days¹⁶. *Alvin* collected core samples on four apparently active mounds on 28 November and 14–15 December 1984; these cores were horizontally sectioned and analysed for excess ^{234}Th . Vertical profiles from the mounds (Fig. 3) show excess ^{234}Th to depths of 2–3 cm (excluding sub-surface peaks from burrow 'piping'¹⁷). Two profiles (1499 and 1500-2) also show nearly constant ^{234}Th excess within vertical depth intervals of 2 cm (Fig. 3); we interpret these as representing episodes of faecal production, during which 1–2 cm of sediment was rapidly deposited on top of the mound. Assuming relatively constant ^{234}Th activity (over time) in surface sediments consumed by echiurans, an advection model for sediment accumulation¹⁶ yields mound deposition rates (w) of ~1–2 cm per month (Fig. 3). The deposition rates determined from the model represent upper limits, because diffusive mixing may contribute to the penetration depth of the excess ^{234}Th signal. X-radiographs of mound sediments cored by *Alvin* reveal few bioturbation structures, however, suggesting that diffusive sediment mixing is unlikely to have significantly affected the penetration of excess ^{234}Th into mound sediments.

The high deposition rates on mounds can be viewed in a basin-wide context by roughly calculating the population-level reworking rates of the echiurans. A photo-sled survey¹⁸ made on 23 November 1984 indicates active (steep-sided) mound densities of ~0.14 m⁻²; if the mounds are 10 cm high, 30 cm wide, and are covered by 1 cm of new faecal material per month, echiurans are processing sediment at the rate of ~1,500 ml m⁻² yr⁻¹. Thus, the top 10 cm of sediment in the SCB can be turned

over by echiurans in ~70 yr; or, in other words, a sediment particle will, on average, pass through an echiuran gut ~10 times before permanent burial¹³. Consequently, while echiuran mounds are rare enough to be poorly sampled by corers from surface vessels, they are likely to have dramatic effects on sediment mixing depths and diagenetic processes at the SCB floor^{14,19}.

The high deposition rates on mounds seem likely to cause major disturbances of the sediment-dwelling community. Deposition rates of 1–2 cm per month cause significant disturbance of intertidal benthos, especially if sediment accumulation is episodic^{20,21}; it seems reasonable that infauna from low-energy, deep-sea environments should be at least as sensitive²². To assess the effect of an episode of mound building on the SCB infauna, we created artificial mounds devoid of macrofauna. This was done by placing recently obtained sub-surface basin sediment into a conical mould; *Alvin* was then used to deposit these sediment cones (~10 cm high and 45 cm in diameter) on the sea floor on 15–16 December 1984. *Alvin* returned ~50 days later and collected core samples for macrofauna on two artificial structures, and from the surrounding, level sea floor (Fig. 4). We collected core samples from portions of the artificial mounds that were 4–10 cm in height; that is, from areas where the original seafloor community was buried to depths of 4–10 cm.

Macrofaunal abundance and species richness on artificial mounds in place for 50 days were unexpectedly high, attaining levels 52–85% of those in background community samples (Fig. 4). The species composition of mound samples was also surprisingly similar to those from the background community; >95% of the animals collected on mounds belonged to species present in background community samples. In addition, the three most abundant species in artificial mounds were among the four most abundant in background samples; in each case

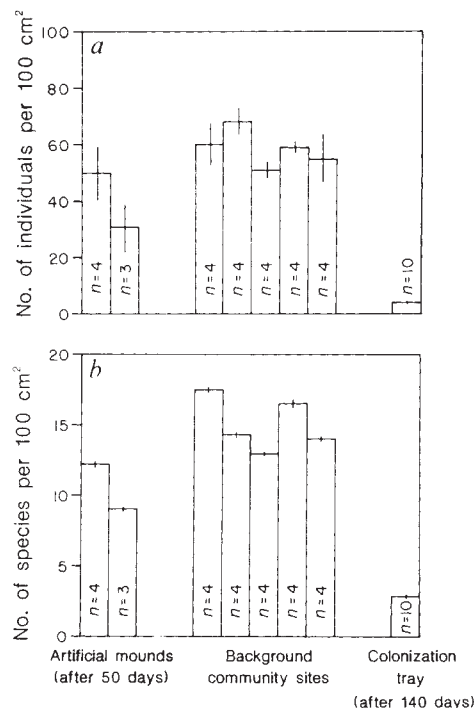


Fig. 4 Mean ($\pm$ s.e.) abundance (a) and species richness (b) of macrofauna (>420 μm) from replicate 100-cm² core samples (n = number of samples) collected from two artificial mounds (left), five locations in the background community ≥ 70 cm from the nearest mound (middle), and a colonization tray of pre-frozen sediment placed on the sea floor for 140 days²⁷ (right).

Methods. Cores sampled 100 cm² of sediment surface area to a depth of 10 cm. Samples from artificial mounds and the background community were collected using *Alvin* and processed as in ref. 6.

Colonization-tray data are from ref. 27.

Table 1 Species contributing $\geq 1.9\%$ of the individuals in two sets of artificial mound cores, and five sets of background community cores

Artificial mounds ($n = 2$)		Background sites ($n = 5$)	
<i>Levinsonia oculata</i>	$68 \pm 2\%*$	<i>Levinsonia oculata</i>	$67 \pm 1\%$
<i>Tharyx monilaris</i>	$5.4 \pm 3.4\%$	<i>Tharyx tessellata</i>	$3.9 \pm 0.5\%$
<i>Cossura</i> sp.	$5.1 \pm 1.8\%$	<i>Cossura</i> sp.	$3.2 \pm 0.7\%$
Tanaid sp.	$1.9 \pm 0.4\%$	<i>Tharyx monilaris</i>	$2.0 \pm 0.5\%$

* Mean $\pm$ standard error.

these three species accounted for $>72\%$ of total macrofaunal abundance (Table 1).

There are two reasonable explanations for the abundance of macrofauna in artificial mounds after 50 days. The first is that the normal SCB assemblage is resistant to burial, with many of its species able to burrow upward through 4–10 cm of rapidly deposited sediment. If this is true, these deep-sea organisms seem better able to withstand burial than many shallow-water species from more physically active environments^{20,21,23,24}. Alternatively, the presence of macrofauna in artificial mounds may result from colonization, arising through immigration from surrounding sediments or recruitment from the water column. If colonization is responsible, the implied rates of community recovery are at least an order of magnitude faster than obtained in earlier deep-sea studies of colonization^{25–27} (Fig. 4). Such differences in colonization rate could well result from biases introduced by the design of earlier experiments, which isolated sediments from the surrounding sea floor in elevated trays^{6,7}.

Whether explained by upward burrowing or by colonization, the abundance of macrofauna in artificial mounds indicates an unexpectedly rapid response in a deep-sea community²². Many SCB species are thus adapted to respond to frequent disturbance resulting from echinuran mound building. In view of the widespread occurrence of echinurans^{9,12} and other mound builders^{3,4} at bathyal depths, a variety of deep-sea communities may be similarly adapted. These findings could affect predictions of the impact of human activities, such as waste disposal or manganese nodule mining, on deep-sea communities.

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End-Cretaceous devastation of terrestrial flora in the boreal Far East

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A continuous marine sedimentary sequence spanning the Cretaceous/Tertiary (K/T) boundary has been identified in eastern Hokkaido, Japan, on the basis of planktonic foraminifera. The K/T boundary is marked by a 6–10-cm-thick layer of pyrite-rich, fossil-poor, greyish-black claystone¹, which is lithologically similar to the well-known 'boundary clay' of the classical European K/T boundary sections^{2–4}. We show here that terrestrial palynomorphs from the Hokkaido section record sudden changes in the floristic composition precisely at the base of the boundary claystone layer. In particular, both angiosperm and gymnosperm pollen abundances decline markedly and these changes are accompanied by an abrupt rise in the proportion of fern spores. Return to the original, moderate level of fern dominance occurs within a few centimetres above the boundary layer. A sharp increase of fern spores has also been reported at the palynologically defined K/T boundary in the western interior of North America⁵, which coincides with the top of an iridium-rich clay layer^{6,7}. The possibly synchronous occurrence of analogous floral changes at these widely separated regions implies a devastation of the land flora which, although geologically brief, was intercontinental in scope, and which was probably caused by a major catastrophic event such as the impact of a massive meteorite⁷.

Although there is general agreement that the great worldwide extinction event of marine floras and faunas signalled the end of the Mesozoic Era, there is less agreement as to whether terrestrial taxa were synchronously affected by the extinction event^{8,9}. To test the model of universal biotic catastrophe resulting from the impact of an asteroid⁷, evidence from land plants is particularly critical because plants form the base of the terrestrial food chain. Palynomorphs, which are generally more abundant in and easier to recover from sediments than macrofossils, should provide a detailed picture of terrestrial change across the era boundary. Unfortunately, palynomorph records published to date do not offer the degree of time-stratigraphic resolution possible for the marine sequence because of the incompleteness of terrestrial sections, and the strong emphasis placed on selected guide fossils rather than characterization of the entire assemblage¹⁰. The study of Tschudy *et al.*⁵, based on

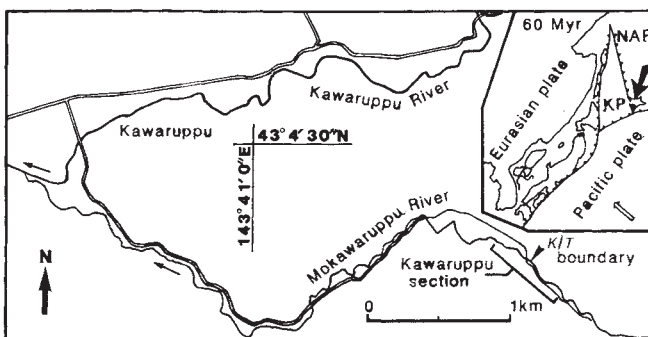


Fig. 1 Location of the K/T boundary section in eastern Hokkaido, Japan, plotted on an early Tertiary palaeogeographic map of the Japanese islands¹⁷. The section studied was deposited on a western continental slope of 'East Hokkaido Island'. Post-Palaeocene subduction of the Kula plate (KP) subsequently joined this island to the main Hokkaido Island, creating the present-day Hokkaido.

non-marine sequences in the western interior of North America, revealed an abrupt but temporary increase in the ratio of fern spores to angiosperm pollen at the palynologically defined K/T boundary. This non-marine K/T boundary lies at the top of a clay layer containing anomalously high concentrations of iridium. An analogous geochemical imprint associated with the K/T boundary has been identified at more than 50 localities worldwide, including marine as well as non-marine sequences¹¹.

A continuous stratigraphic section spanning the K/T boundary was identified in the eastern district of Hokkaido, northernmost Japan (Fig. 1), based on planktonic foraminiferal analyses¹. The K/T boundary occurs in the upper part of the Katsuhira Formation, which consists predominantly of a massive, dark-grey siltstone bearing occasional calcareous concretions. Benthic foraminifera in the siltstone facies indicate that the sediments were deposited in upper- to mid-bathyal water depths. The upper Katsuhira sequence yields *Rugoglobigerina*-dominated assemblages of latest Maastrichtian age, which are in turn overlain by a 2.6-m-thick basal Danian bed containing *Globigerina fringa*, *Globigerina eugubina* and *Globigerina minutula* (Fig. 2). A 6–10-

cm-thick, greyish-black claystone layer separates the latest Cretaceous sequence from the earliest Tertiary bed. Framboidal pyrite abounds in the claystone and iron is five times more concentrated than in sediments above and below (Fig. 3). The occurrence of this anomalous claystone coincident with the K/T boundary is strongly reminiscent of the boundary clay layer that characterizes the classical K/T boundary sequences of Europe^{2–4}.

Palynofloras contained in the Hokkaido section provide, for the first time, an opportunity for examining changes in terrestrial floras directly keyed to the marine extinction event. Three distinct palynomorph assemblages characterize the Hokkaido section, in upward sequence (Fig. 3): (1) fern-angiosperm-rich floras of the latest Cretaceous interval; (2) impoverished palynomorph assemblages, dominated by fern spores, in the boundary claystone; and (3) pine-dominated gymnosperm pollen in the earliest Tertiary interval. The most distinct floral change occurs at the base of the boundary claystone, where both angiosperm and gymnosperm pollen abundances show a marked decline and fern spores increase dramatically. The preservation state of palynomorphs also deteriorates notably in the boundary layer, where dark, charcoal-like woody tissues are commonly observed.

Pollens of the Aquilapollenites group, angiosperm pollen grains of unusual morphology, were found by Orth *et al.*⁶ to disappear at the K/T boundary in the western interior of North America, but at Hokkaido this pollen group, although representing <3% of the floras, continues to the Palaeocene. In fact, this pollen group ranges up to the Eocene in the Fushun and Hubei Provinces of eastern China¹². Although the Aquilapollenites group is not age-diagnostic in Asia, their presence suggests that eastern Hokkaido and the western interior of North America belonged to the same floral province. During the mid- to late Cretaceous, the pollen flora of the mid- to high-latitude Northern Hemisphere gradually diverged into two distinct provinces, the boreal Aquilapollenites and southerly Normapolles provinces^{10,13}.

A few centimetres above the boundary claystone, the sediments become rich in palynomorphs and fern percentages drop to dominance levels characteristic of the Cretaceous interval (Fig. 3). The Palaeocene floral complex was, however, distinctly different from the Cretaceous flora, in that the *Pinus* pollen content increased by a factor of 3–4.

The pattern of palynological changes at the K/T boundary is identical in eastern Hokkaido and the western interior of North America, and strongly indicates the same causal mechanism. Tschudy *et al.*⁵ advanced a model of extensive devastation of forested terrains at the end of the Cretaceous. The discovery¹⁴ of a layer of soot at the K/T boundary sites in Denmark, Spain and New Zealand led Wolbach *et al.*¹⁴ to postulate that a major wildfire set off by a meteorite impact burned down much of the Earth's Cretaceous vegetation and/or ignited substantial amounts of fossil fuels. During the deposition of the boundary layer, few palynomorphs were incorporated in the sediment because of the general scarcity of living land plants, possibly coupled with rapid sedimentation. The persistent high iron content characterizing the boundary clay attests its deposition as a coherent unit, and the near absence of benthic foraminifera¹ is attributable to a high sedimentation rate. Ferns were the first to re-establish themselves on the wasted terrains, because they had ecological advantages over tree species, deriving from their short life cycle and high reproductive fitness (tolerance to soil deficient in mineral nutrients, wind dispersal of spores and ability to start new growth from rhizomes and root systems). After the temporary dominance of ferns, pines invaded rapidly, and were subsequently followed by angiosperm species, which survived the devastation in widely scattered refuges. Pine species are well known as pioneers of reforestation^{15,16}, because pine seedlings are adapted to the brightly lit and sometimes dry-soil conditions of deforested land.

Thus, the idea of wildfires at the end of the Cretaceous,

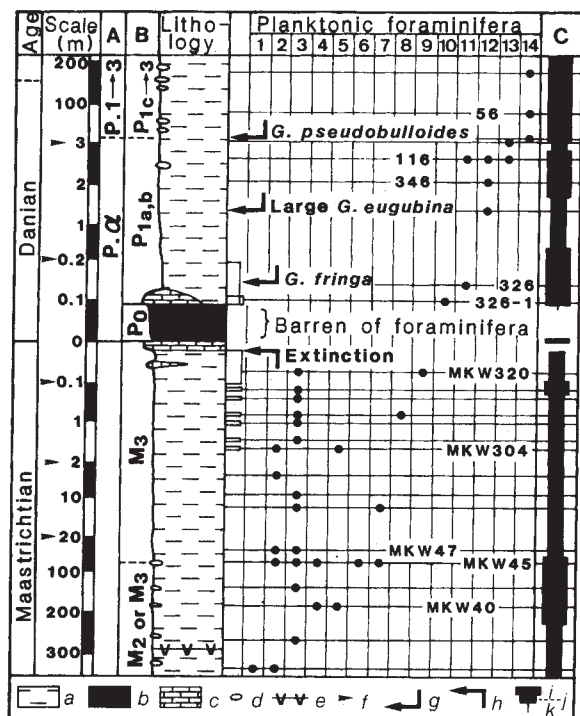
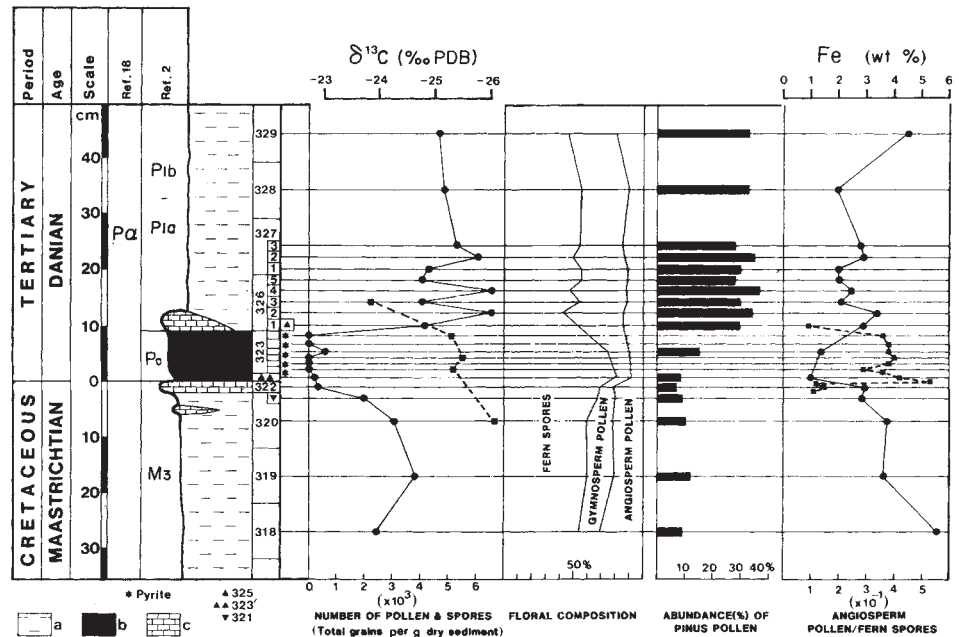


Fig. 2 Lithology of the K/T boundary interval in the Kawaruppu section, eastern Hokkaido. Stratigraphic ranges of planktonic foraminifera are plotted relative to the zonation of refs 18 (A) and 2 (B); fluctuations in the abundance of benthic foraminifera are also shown (C). Lithologies: a, siltstone; b, claystone; c, limestone; d, calcareous concretions; e, tuff layer. Arrows f mark the locations of scale changes for sediment thickness; g, the entry of age-diagnostic Palaeocene planktonic taxa; h, the extinction level of Cretaceous taxa. Benthic foraminifera abundances: i, between 1,000 and 500 specimens per 100 g dry sediment washed on 150-mesh screen; j, between 499 and 250; k, one specimen only. Planktonic species symbols: 1, *Globigerinelloides multispinosa* (Lalicker); 2, *Rugoglobigerina hexacamerata* Bronnimann; 3, *R. rugosa* (Plummer); 4, *R. milamensis* Smith and Pessagno; 5, *R. macrocephala* Bronnimann; 6, *Globotruncanella kefennsoura* Solakius; 7, *Globotruncanella petaloidea* (Gandolfi); 8, *Rugoglobigerina scotti* (Bronnimann); 9, *Globigerinelloides volutus* (White); 10, *Eoglobigerina* sp., type II of ref. 18 (plate 60); 11, *Globigerina fringa* Subbotina; 12, *Globigerina eugubina* Luterbacher and Premoli Silva; 13, *Globigerina minutula* Luterbacher and Premoli Silva; 14, *Globorotalia pseudobulloides* (Plummer).

Fig. 3 Stratigraphic section spanning the K/T boundary in eastern Hokkaido, with the boundary drawn at the base of a thin, greyish-black claystone occurring in a dark-grey siltstone sequence. The claystone is accompanied by thin limestone beds immediately above and below. Lithological symbols as for Fig. 2. Total palynomorph grains per g dry sediment, floral composition and ratios between angiosperm pollen and fern spores are shown by solid lines. Histograms give *Pinus* pollen percentages relative to the total palynomorph assemblage. Dashed lines with squares show results of two geochemical analyses, $\delta^{13}\text{C}$ values of total organic carbon (left-hand column) and abundance of iron as determined by instrumental neutron activation analysis (right-hand column).



triggered by a giant meteorite impact, can explain both the pattern of floral change across the K/T boundary and the pine-dominated climatic vegetation which followed the end-Cretaceous event in the boreal Far East.

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Spatial heterogeneity: evolved behaviour or mathematical artefact?

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For more than a century ecologists have sought to explain the spatial heterogeneity of plants and animals¹, but progress has been hampered by measurement bias². A measure thought to be an unbiased index of spatial heterogeneity³ is b , the fitted exponent in the empirical relationship $s^2 = aM^b$, where s^2 is the variance and M the average of randomly placed replicate population estimates⁴. This index is widely accepted because of the impressive correlation between s^2 and M and because it requires no inter-organism distance measures. Theoretical models, based on migratory behaviour⁵ or demographic factors⁶, have been proposed to account for the relationship between s^2 and M . These models disagree regarding the effect of environment on b and the divergence of b values shown by different species. Here I report data showing that species-specific b varies among environments and that different species often show similar b values, favouring the demographic model. Analysis of these data shows that different

levels of replication and sampling coverage lead to biased b values, casting doubt on the use of b for the deduction of cause of spatial variance relationships or the comparative measurement of spatial heterogeneity.

Plants and animals are not uniformly distributed in nature. Two contrasting mechanisms^{5,6} have been proposed to explain the empirical power-function relationship between s^2 and M (ref. 7). One mechanism suggests that the fitted exponent b is a measure of spatial heterogeneity that results from a specifically evolved combination of 'migratory' and 'congregatory' behaviours⁸⁻¹⁰ (the Δ model). The second suggests that b arises from the stochastic interplay of demographic characteristics of populations and environmental heterogeneity^{6,11-13}. These models disagree on at least three points. First, demographic simulations suggest that b values are highly variable among populations in different growth phases or environments⁶, whereas the Δ model suggests that b is constant within species regardless of environment⁵. Second, the Δ model suggests that because b results from innate behavioural responses, b values 'segregate' species^{3,14}. Therefore, b fitted for different species should frequently be dissimilar. Third, demographic models considering environmental heterogeneity show that b should rarely lie outside the range 1-2 (ref. 6). The Δ model allows greater latitude in b ; it is supported by fitted values as high as 3.9 (ref. 15). Next I present tests of these controversial points.

Discussion surrounding one of the earliest estimations of a s^2 : M power-function¹⁶ suggests that b is an intrinsic property

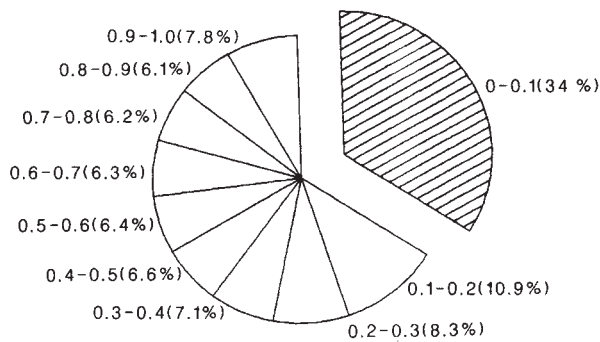


Fig. 1 Frequency of probability levels obtained in 24,310 pairwise *t*-tests of the hypothesis that species-specific *b* values are equal. Comparisons are for 221 species of aquatic and terrestrial animals (refs 17–37 and refs listed in ref. 29 as 3, 5, 6, 9, 10, 16, 18, 19, 21, 29–31, 38–40, 43, 44, 48, 55, 56, 59, 62, 67, 70, 74, 75, 81, 90, 94, 97 and 101). Variance functions were calculated on a per sampler basis (unstandardized to area or volume), and include only organisms from a single sampling site. Analyses include only species for which correlations between $\log s^2$ and $\log M$ were statistically significant ($P < 0.01$).

of the organisms concerned and therefore independent of environmental influence. Previous tests of this hypothesis have been anecdotal⁵ or confound temporal and spatial variability (for example, ref. 14). Inter-habitat comparisons of *b* values estimated by repeated sampling of several species of zooplankton, benthic invertebrates and terrestrial insects (Table 1) reveal frequent dissimilarity of *b* values within species. I found significant differences among *b* values ($P < 0.05$) in 86% of species. Of 19 possible intra-specific comparisons, 30% show different values of *b* in replicate determinations. The spatial heterogeneity of species, as measured by *b*, varies significantly among environments.

The view that *b* arises because of species-innate behavioural responses is essential to the Δ model and is inconsistent with a stochastic/demographic approach. The evolutionary origin of behavioural responses suggests both their constancy within species and their individuality^{5,8}. Proponents of the Δ model believe that, given enough statistical power, most species could be demonstrated to have significantly divergent *b* values¹⁴. Frequency histograms of *b* values have been published^{4,5,15}, but nobody has examined these exponents to find the frequency with which the null hypothesis of equality of *b* values can be rejected.

I collected observations of *M* and s^2 from several publications (see Fig. 1 legend) and performed linear regression analyses of $\log s^2$ on $\log M$ specific to species, site and design (sampler and replication level) for 221 species of terrestrial and aquatic animals (data available from me on request). Despite the strength of correlations (77% of r^2 are > 0.8) and the precision of *b* values (78% of s.e._{*b*}/*b* are < 0.2), dissimilar *b* values are rare. Figure 1 shows the distribution of *t* probability levels for all possible pairwise tests of the null hypothesis. Only about 1/3 of the comparisons show significant differences ($P < 0.1$) and 30% of these would be expected by chance alone. The null hypothesis of equal *b* values can therefore be accepted with $P > 0.1$ in at least 2/3 of the pairwise comparisons of *b*. Less than 2% of the taxa examined yield *b* values different ($P < 0.05$) from all other species. Either there is a high degree of convergence in the evolution of species behaviour or stochastic, mathematical or demographic factors are operating similarly on many species.

Monte Carlo simulations of the stochastic/demographic model of spatial distribution with moderate environmental heterogeneity⁶ predict that *b* should vary only between 1 and 2, with exact values determined by population parameters and environmental variation. This contrasts with empirical studies that show values of *b* ranging between 0.4 and 3.9 (ref. 5). Such

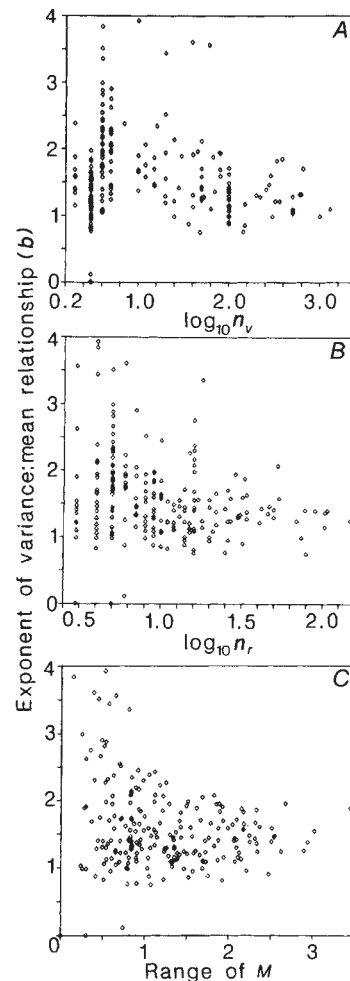


Fig. 2 Site, species and design-specific *b* values from published sources (see Fig. 1 legend), plotted against: A, the $\log_{10}$ of the number of replicate population estimates included in *M* and s^2 (n_v); B, the number of (*M*, s^2) pairs included in each variance regression (n_r); and C, the range of *M* in logarithmic form ($\log_{10} M_{\max} - \log_{10} M_{\min}$) covered by each variance regression. Two negative values of *b* have been omitted from the extreme left of each plot. Variance functions were calculated on a per sampler basis (unstandardized to area or volume), and include only organisms from a single sampling site. Analyses include only species for which correlations between $\log s^2$ and $\log M$ are statistically significant ($P < 0.01$).

studies use a wide range of replication both in calculation of *M* and s^2 , and in the estimation of regression coefficients. Greater replication in *M* and s^2 calculations (n_v) would lead to more accurate estimates of μ and σ^2 and less error in the fit of regressions^{38–40}. Inclusion of more (*M*, s^2) pairs (n_r) in regressions would also lead to more stable estimates of *b* (ref. 41).

Contrary to current opinion³, *b* is biased by mathematical artefact. The range and size of *b* vary with the number of samples taken and the range of *M* considered. The range of *b* is largest where n_v , n_r , or the range of *M* covered by the s^2 : *M* relationship is small (Fig. 2A–C): *b* outside the range of 1–2 is rare where *M* and s^2 are calculated on > 60 replicates (Fig. 2A); more than 20 (*M*, s^2) pairs are included in the regression (Fig. 2B); or more than one order of magnitude is covered by *M* (Fig. 2C). Extreme values of *b* ($b < 1$ or $b > 2$) appear frequently at low replication or narrow density coverage. Stable and robust estimates tend to $1 < b < 2$ as suggested by the demographic model⁶.

I found that *b* varies systematically with degree of replication. A multiple regression analysis (three negative *b* values omitted where *M* spanned 0.0001 of an order of magnitude) shows that

Table 1 Student's *t*-tests of replicate species-fitted *b* values

Species/sampling site	Ref.	n_v	n_r	b	s.e. _{<i>b</i>}
<i>Bosmina coregoni</i>					
Donk Lake, Belgium	17	39	6	3.61	0.608
Belgian reservoir	18	5	6	2.23	0.093
<i>Leptinotarsa decemlineata</i>					
Field near Chatham, Ontario	19	144	16	0.76	0.066
Plots near Merivale, Ontario	20	5	16	1.40	0.365
<i>Limnodrilus hoffmeisteri</i>					
Lake Norman, North Carolina	21	2	5	1.88	0.178
Lake Norman, North Carolina	21	3	26	1.23	0.083
Lake St Clair, Michigan/Ontario	22	2	7	1.15	0.289
Lake St Clair, Michigan/Ontario	22	3	78	1.40	0.092
<i>Monodiamesa bathyphila</i>					
Lake St Clair, Michigan/Ontario	22	3	4	0.83	<0.001
Harding Lake, Alaska	23	3	3	1.40	<0.001
<i>Papillia japonicum</i>					
Plots near Jacobstown, New Jersey	24	25	4	2.14	0.157
Plots near Jacobstown, New Jersey	24	50	4	2.12	0.221
Plots near Jacobstown, New Jersey	24	624	8	1.31	0.212
<i>Pieris rapae</i>					
Plots near Merivale, Ontario	25	5	16	2.75	0.741
Field near Sendai, Japan	26	50	11	1.23	0.087
<i>Pyrausta nubilalis</i>					
Field in north-east Iowa	27	324	3	1.21	0.003
Field in north-east Iowa	27	1296	3	1.09	0.011
Fields in Wood County, Ohio	28	4	21	1.08	0.240
Fields in Wood County, Ohio	28	10	16	1.69	0.327

Significant differences between *b* values at: $P < 0.05$ (*), $P < 0.01$ (**). n_v , number of replicate estimates included in each calculation of M and s^2 ; n_r , number of (M , s^2) pairs in each regression analysis; s.e._{*b*}, standard error of the estimate of *b*.

$b = 2.057 - 0.11 \log_{10} n_v - 0.37 \log_{10} n_r$ (218 species; $F = 8.2$; $P < 0.001$) despite heteroscedasticity. Bias in *b* is a major technical problem, suggesting that comparisons of *b* should only be made where identical levels of replication are used and equivalent ranges of *M* are examined.

Concrete examples of the bias in *b* are shown in Table 1. *Papillia japonicum* and *Pyrausta nubilalis* have significantly different *b* values although determinations of *b* were made on the same sites and dates. The lower value of *b* is always found where more samples are analysed. Hence, competing theoretical models are arguing about the ecological meaning of mathematically biased estimators of spatial heterogeneity, rather than about spatial heterogeneity itself.

Environmental differences, demographic factors, evolved behaviour and statistical artefact may each play a part in the perceived spatial distribution of natural populations. Analysis of *b* values of spatial variance relationships suggest that spatial heterogeneity is no less variable within species than among species. The assumptions of the demographic model therefore seem most realistic. Unfortunately, *b* values are biased and cannot be used as support or criticism of any causal model of spatial distribution.

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Inhibition of microbial activity in marine sediments by a bromophenol from a hemichordate

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Allelochemicals, a class of organic compounds, affect succession, competition, predation and other interactions between organisms¹⁻³. Bromophenols, a member of this class, are found in marine algae and invertebrates, particularly annelids, phoronids and hemichordates^{4,5}. Bromophenols are toxins with bacteriocidal properties⁶⁻⁹. Here I report inhibition of microbial activity in marine sediments by the common bromophenol, 2,4-dibromophenol (DBP), from the hemichordate *Saccoglossus kowalewskii* and demonstrate that anaerobic microbial metabolism in sediments is relatively unaffected by DBP whereas aerobic metabolism is particularly sensitive. These data, together with observations of the distributions of DBP and burrow-wall chemistry, suggest that secretion of DBP inhibits the aerobic microbial degradation of the burrow-wall mucous lining or alters local biogeochemistry. Thus, bromophenols may be targeted against higher organisms with which *S. kowalewskii* competes as well as against microorganisms.

Table 1 DBP concentrations in ether extracts of various Lowes Cove intertidal fauna

Organism	Concentration
<i>S. kowalewskii</i>	9.9 (2.2)
<i>Nereis succinea</i>	0.014
<i>Glycera</i> spp.	ND
<i>Clymenella</i> spp.	ND
<i>Tharyx</i> spp.	ND

Concentrations in $\mu\text{mol per g}$ fresh weight (± 1 s.e.; $n = 3$); ND, not detected. DBP was analysed by extracting homogenized tissues with ether. The extracts were diluted as necessary with methanol and 1–2 μl injected into a Varian 3700 gas chromatograph fitted with a ^{63}Ni electron-capture detector and a wide-bore capillary column (0.53 mm inner diameter $\times$ 15 m) containing 5% phenylmethylsilicone. The column was operated at 150 $^{\circ}\text{C}$ with a carrier gas of N_2 flowing at 10 ml min^{-1} . The identity of DBP was determined by comparison with known standards and by gas chromatography–mass spectroscopy for worm tissue extracts.

S. kowalewskii is a relatively common hemichordate occurring along the eastern coast of the United States¹⁰. Specimens obtained from Lowes Cove, Maine, contain relatively high concentrations of DBP (Table 1). A related organism, *Balanoglossus biminiensis*, is reported to contain 2,6-dibromophenol in similar concentrations¹¹. Other hemichordates also contain bromophenols or halo-indoles^{4,5,9}, but high concentrations of halogenated phenols are not a general characteristic of the sediment biota. Concentrations of halogenated phenols in several polychaetes common in Lowes Cove are at trace or non-detectable levels (Table 1).

To determine the effects of DBP on microbial activity, I measured glucose oxidation in sediments from an area with dense populations of *S. kowalewskii*. DBP was added at final concentrations similar to those in the mucous lining and inner burrow-wall sediments (10–1,000 nmol cm^{-3}). Results show that DBP has only a limited effect on glucose oxidation in anaerobic sediments (Fig. 1a). Inhibition of oxidation is observed at 1,000 nmol cm^{-3} in all treatments, but the inhibition lessens over time and is ameliorated by the addition of glucose. In particular, the addition of glucose results in only slight inhibition after 12 h ($<20\%$); preincubation also significantly lessens inhibition. Only slight inhibition is observed at 100 nmol cm^{-3} and none is found at 10 nmol cm^{-3} (data not shown).

Glucose oxidation in the aerobic treatments is inhibited to a much greater extent than in comparable anaerobic treatments (Fig. 1b). Inhibition is particularly marked in sediments that are not preincubated with DBP or that have no added glucose. Preincubation and glucose addition do reduce the inhibition, but to a lesser extent than under anaerobic conditions. In fact, preincubation results in decreased oxidation for up to 12 h after the addition of radio-labelled glucose; however oxidation increases between 12 and 24 h and exceeds that in samples that are not preincubated. Inhibition of glucose oxidation is also observed at 100 nmol cm^{-3} DBP but the effect is relatively small and decreases over time. Even so, inhibition at this level is greater than in anaerobic incubations. No effect is measurable at 10 nmol cm^{-3} (data not shown).

To test the possibility that patterns of aerobic and anaerobic DBP degradation differ, I added DBP to triplicate sediment slurries and then incubated them aerobically or anaerobically. Aliquots of slurry were removed periodically for analysis; slurries sterilized by autoclaving served as controls.

Under aerobic conditions, DBP is degraded slowly with a loss of only 12% after 7 days and 26% after 17 days (Fig. 2). In contrast, DBP disappears rapidly under anaerobic conditions (Fig. 2). Within 1 day, 73% of the added DBP is consumed; degradation continues for several days resulting in a loss of $>97\%$. Losses in the sterile controls are negligible. The time-course of DBP degradation in each of the treatments is consistent with the inhibition patterns observed for glucose oxidation

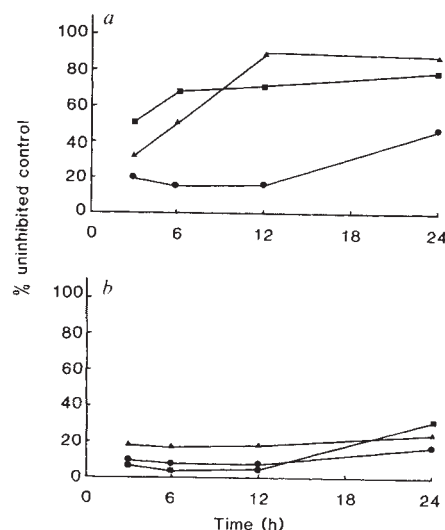


Fig. 1 Inhibition of $[\text{U-}^{14}\text{C}]$ glucose oxidation by DBP (1,000 nmol cm^{-3}); data presented as a percentage of $^{14}\text{CO}_2$ production in uninhibited controls. *a*, Anaerobic; *b*, aerobic. Treatments include addition of DBP only (●), preincubation of slurries with DBP for 14 h before addition of radiolabel (■), and addition of DBP plus 1 $\mu\text{M cm}^{-3}$ glucose (▲); each treatment was compared with controls with or without added glucose as appropriate and no DBP. *a*, Data from slurries incubated anaerobically with 100% N_2 ; *b*, data from slurries incubated aerobically. Sediments for the slurries were collected from the 0–1- and 1–5-cm depth intervals of Lowes Cove for aerobic and anaerobic incubations, respectively. Slurries were prepared by diluting sediments 1:1 with 0.45 μm filtered sea water. 9 cm^3 of slurry was dispensed into test tubes fitted with septum caps and the tubes injected with 0.5 ml of appropriate concentrations of DBP and either 0.5 ml of an aqueous solution containing $[\text{U-}^{14}\text{C}]$ glucose (74 kBq ml^{-1} ; 10.95 GBq mmol^{-1} specific activity, NEN) or 0.5 ml $[\text{U-}^{14}\text{C}]$ glucose plus stable glucose (1 mM final concentration). Production of $^{14}\text{CO}_2$ was determined by acidifying the slurries and trapping the evolved radiolabel in base for liquid scintillation counting. Each time-point represents the mean of a triplicate determination. Error bars are less than the dimensions of the symbols.

(Fig. 1). In fact, the rapid degradation of DBP under anaerobic conditions may explain the observed inhibition of anaerobic glucose oxidation during the first 12 h of incubation (Fig. 1a); bulk substrate metabolism may have switched temporarily from glucose to DBP. The differential sensitivity can be attributed to the potent inhibition of aerobic electron transport^{12,13}.

Analyses of *S. kowalewskii* and associated sediments suggest that the distribution of DBP is limited (Table 2). The highest concentrations are found in the worm tissue (~ 0.25 and 2.0% on a wet and dry weight basis, respectively) and mucus. The highest concentrations external to *S. kowalewskii* are found in the burrow walls; concentrations in burrows >5 cm deep are much lower than in surface burrows (Table 2). A transect away from a surface burrow reveals that DBP concentrations decrease rapidly with distance (Table 2). Within 1–2 mm, DBP concentrations are 38-fold less than in the burrow wall.

Fresh *S. kowalewskii* fecal material contains DBP concentrations considerably lower than in the worm tissue but intermediate between surface burrow-wall, mucous lining and bulk sediments (Table 2). *S. kowalewskii* fecal coils collected *in situ* have concentrations lower than *in vitro* samples, but the material collected *in situ* is probably more representative because the *in vitro* samples are unavoidably contaminated with bromophenol-containing mucus from the worms.

The distribution of DBP and its effect on microbial metabolism suggest several possible functions. Microbial activity may be inhibited along the walls of *S. kowalewskii* burrows. Inhibition in other areas is unlikely because volumetric concentrations are only several hundred pmol to a few nmol cm^{-3} . Although the

Table 2 DBP concentrations in sediments and mucous lining from burrows of *S. kowalewskii*

<i>S. kowalewskii</i>	9,900 (2,200)
Surface burrow walls (from 1–2 cm depth)	71.3 (2.6)
Mucous lining of surface burrow walls	46.6 (3.8)
Deep burrow walls (>5 cm depth)	0.8 (0.1)
1–2 mm from surface burrow walls	1.9 (0.4)
4–6 mm from surface burrow walls	0.1 (0.02)
9–11 mm from surface burrow walls	0.3 (0.02)
'Fresh' fecal coils	26.6 (3.3)
Fecal coils collected <i>in situ</i>	4.4 (0.2)

DBP concentrations in nmol per g fresh weight of sediment or burrow lining (± 1 s.e.).

greatest inhibition for added DBP is at final concentrations $\sim 6\times$ that found in mucus (Table 2), >90% of the added DBP is adsorbed and probably ineffective in inhibition. Thus, the dissolved DBP concentrations most inhibitory in this study are those found in the mucus.

Inhibition of aerobic activity can decrease biological oxygen uptake resulting in a larger zone of chemical sulphide and ferrous iron oxidation^{14,15}. This would decrease the influx of toxic hydrogen sulphide from reduced sediments bordering the burrow. In addition, the oxidation of ferrous iron would result in the deposition of iron oxyhydroxides that provide a barrier to sulphide influx and serve as a 'cement' to stabilize the burrow wall^{16,17}. Indeed, one characteristic of *S. kowalewskii* burrow walls is a highly visible 2-mm-thick deposit of iron oxyhydroxides. This deposit contains about 470 μmol Fe per g dry weight, a value more than twice that of bulk sediment. These effects of *S. kowalewskii* burrows on the local microenvironment may differ markedly from those of other burrowing organisms in Lowes Cove^{18,19}.

Decreased microbial metabolism could also increase the longevity of burrow-wall mucous lining. Although estimates of the energetics of mucus production in *S. kowalewskii* are unavailable, it is clear that a decreased requirement for replacing mucus could result in a greater allocation of energy for growth and reproduction. Secretion of bromophenols into mucus might represent an effective mechanism for inhibiting the decomposition of what otherwise has been regarded as a readily available substrate^{14,15}.

Finally, I note that bromophenols are natural analogues of chlorophenols, a class of common pollutants¹⁸. It has been suggested that analogues of halogenated pollutants are rare¹⁹, but at least some analogues are found in many marine organisms^{4,5} and are of sufficient abundance to play a part in the biogeochemistry of bromine in marine sediments²⁰. A detailed study of the microbiology and ecology of marine bacteria capable of degrading these compounds could be important in

elucidating the metabolism of other halophenols. It may also prove feasible to isolate marine bacteria that are more specifically adapted for halophenol degradation than bacteria found in terrestrial or freshwater systems, where halophenols are not reported to occur naturally²¹. These bacteria could serve as a genetic resource for developing pollutant-degrading bacteria in non-marine systems.

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Immunization of *Aotus* monkeys with recombinant proteins of an erythrocyte surface antigen of *Plasmodium falciparum*

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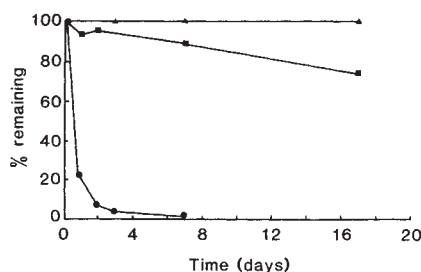


Fig. 2 Time course of added DBP (final concentration 500 nmol cm^{-3}) in sterile controls (▲) and slurries incubated anaerobically in 100% N_2 (●) or aerobically (■); data presented as a percentage of initial concentrations. DBP concentrations were determined by extracting sediments with ether and then analysing the extract by gas chromatography. Each time point represents the mean of a triplicate analysis. Error bars are less than the dimensions of the symbols.

Recent studies have identified and characterized a ring-infected erythrocyte surface antigen (RESA) of the human malaria parasite *Plasmodium falciparum* with a relative molecular mass (M_r) of $\sim 155,000$ (refs 1–7). RESA is localized in the micronemes of merozoites and also the membrane of red cells infected with ring-stage parasites. It is thought to be released through the apical pore from the rhoptry at the time of merozoite invasion⁵. Because antibodies directed against this antigen strongly inhibit parasite growth *in vitro*, RESA may be useful in developing a vaccine against this parasite^{4,7}. Here we describe an immunization trial using *Aotus* monkeys and *Escherichia coli*-derived fused polypeptides corresponding to various regions of the RESA molecule. Some monkeys in all test groups, but not in the control group, were protected against overwhelming infection. Strikingly, protection correlated with antibody responses to either of two different repetitive sequences in RESA.

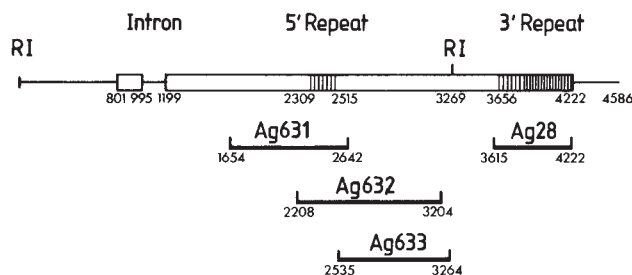


Fig. 1 Predicted structure of the RESA gene and segments that have been expressed in *E. coli*. The structure shown consists of the sequence of a cloned chromosomal *EcoRI* fragment from *P. falciparum* isolate FCQ27/PNG (FC27) (nucleotides 1–3,269) joined at the internal *EcoRI* site to the sequence of a cloned FC27 cDNA (Ag46, nucleotides 3,270–4,586) that has been described previously². The relative positions and end-points of fragments expressed in *E. coli* and used to vaccinate *Aotus* monkeys are shown.

Methods. The chromosomal *EcoRI* fragment was cloned in λ gt10 and detected with NF7 Ag13L derived from cDNA clone NF7 Ag13 (overlapping the internal *EcoRI* site²). All clones were sequenced in full by the chain-termination procedure¹². NF7 Ag13 (derived from isolate NF7) is colinear with the sequence shown from nucleotide 1,654–3,727; 3' to this it has probably undergone deletions in *E. coli*². The fragments expressed in *E. coli* (Ag631, Ag632 and Ag633) were generated by sonicating the 5' *EcoRI* fragment (nucleotides 1,654–3,269, these correspond to nucleotides 1–1,616 in ref. 2) of clone NF7 Ag13. The fragments were ligated into λ gt11-amp3 after the addition of linkers (GGAATTC) and screened by hybridization with fragments of NF7 Ag13 to determine which parts of the sequence were present. The probes used were from the *EcoRI* linker to the *PstI* site (nucleotides 1,654–1,969); a 352-base pair (bp) *AluI* fragment (2,162–2,513) and a 485-bp *HincII-EcoRI* fragment (2,785–3,269). Clones bearing different portions of the RESA gene were tested for production of stable fused polypeptides by electrophoresis of lysates from the induced clones on SDS-polyacrylamide gels, followed by staining with Coomassie blue¹³. Clones Ag631, Ag632 and Ag633 were selected because they contained a set of overlapping fragments and produced substantial amounts of stable fused polypeptides. The exact end-points of these three fragments were determined by sequencing. Ag631 contains a 989-bp insert (nucleotides 1,654–2,642) and therefore encodes 218 amino acids 5' to the repeats as well as the 5' repeats. Ag632 contains a 997-bp insert (2,208–3,204) and therefore encodes the 5' repeats. The 5' *EcoRI* site in this clone was destroyed by a cloning artefact; the 5' overhang of the vector *EcoRI* site was apparently removed and nucleotide 2,208 of the blunt-ended fragment ligated directly to the resulting blunt end. The sequence was therefore determined on a *SacI-EcoRI* fragment spanning the fusion point using a synthetic oligonucleotide corresponding to the β -galactosidase sequence. Ag633 contains a 730-bp insert (nucleotides 2,535–3,264) and therefore is located downstream from the 5' repeats. The complementary DNA clone Ag28 from isolate FC27 extends from nucleotide 3,615 to 4,222 (ref. 2) and produces a fused polypeptide containing the 3' repeats¹³. For the isolation of fused polypeptides, the appropriate bacterial cells were grown to mid-logarithmic phase in aerated 800-ml cultures, heat-induced at 45 °C for 15 min and incubated for a further 90 min at 37 °C. The bacterial cells were lysed by treatment with 0.25 mg ml⁻¹ lysozyme, 10 mM Tris, pH 8, 2 mM EDTA and 50 mM NaCl. Triton X-100 was added to a final concentration of 0.2% and the solution was made to 10 mM MgCl₂ and 1 μ g ml⁻¹ DNase. After incubation for 30 min at room temperature, cell debris was removed by centrifugation at 850 g. Insoluble bacterial proteins were collected by centrifugation for 20 min at 50,000 g, then solubilized in 0.1 M phosphate buffer, pH 6.8, containing 2% SDS and 10 mM dithiothreitol (DTT). The solubilized proteins were then fractionated by size-exclusion chromatography on a Sephacryl S300 column (25 $\times$ 90 mm) linked in series with a Sephacryl S400 column (25 $\times$ 90 mm). The columns were equilibrated with 0.1 M phosphate buffer, pH 6.8, containing 0.1% SDS and 1 mM DTT at a flow rate of 40 ml h⁻¹. Small samples of each fraction were electrophoresed on SDS-polyacrylamide gels to identify the elution position of the fused polypeptides. Fractions most enriched for fused polypeptides were pooled. The fused polypeptides were concentrated from this solution and freed of ~90% of contaminating SDS by adsorption into hydroxyapatite (HA). For this the pooled fractions were mixed for 20 min at room temperature with HA which had been washed in 0.01 M phosphate buffer, pH 6.8, containing 0.1% SDS and 1 mM DTT. Subsequently, the HA was allowed to settle, the supernatant discarded and the HA resuspended in 0.1 M phosphate buffer, pH 6.8, containing 1 mM DTT but no SDS. After the HA was packed into a 200 $\times$ 15 mm column, fused polypeptide was eluted with 0.5 M phosphate buffer, pH 6.8, containing 1 mM DTT. The fused polypeptides were stored in this solution at -70 °C until used.

The complete structure of the gene encoding RESA has recently been determined (see Fig. 1). An intron in the gene separates a small exon 1 (65 amino acids) from a very much larger (1,008 amino acids) exon 2. Within exon 2 are two regions of repetitive sequences: the 3' repeat region encodes several tandem repeats of an 8-amino-acid sequence (EENVHDA, single-letter code) followed by a much more extensive set of tandemly repeating 4-amino-acid sequences (predominantly EENV). The 5' repeat region is more degenerate with an 11-amino-acid sequence (DDEHVEEPTVA) occurring twice and 5 shorter sequences derived from the 11-mer by deletions and in some cases conservative substitutions².

The purpose of our experiment was to determine the efficacy of recombinant proteins containing elements of the RESA molecule for inducing protective immunity in monkeys susceptible to infection with *P. falciparum*. We randomly assigned 20 Peruvian *Aotus* monkeys of karyotypes V, X and XI to a control and three vaccine groups, five animals per group. Two animals from the control group and one from the vaccine group II died before challenge. Following immunizations given 56 and 14 days before challenge, each monkey was inoculated intravenously with 1×10^6 asexual parasites from a donor *Aotus* monkey infected with the Indochina I/CDC strain of *P. falciparum*^{8–10}. Parasitemias were monitored daily by microscopic examination of thick and thin blood films stained with Giemsa stain¹¹. Before the trial began, protection was defined as survival with maximum parasitemia of <10% of the red blood cells infected.

Serum samples collected on the day of challenge were examined by indirect immunofluorescence assay (IFA) with air-dried blood films and blood films wet-fixed with glutaraldehyde and air-dried (RESA-IFA)¹. Enzyme-linked immunosorbent assays (ELISAs) were performed on the same sera using three different synthetic peptides corresponding to different repetitive sequences in the 3' and 5' regions of the RESA molecule.

The immunization and challenge studies (Fig. 2) demonstrate protection in 9 of the 14 animals in the vaccine groups. The Indochina I/CDC strain of *P. falciparum* is usually lethal to Peruvian monkeys of the karyotypes used and would be expected to produce parasitemias of $\geq 10\%$ in animals inoculated with 1×10^6 parasites. Of the three control animals that were challenged, two animals, AO-411 and AO-419, were treated after their parasitemias rose rapidly to >10%. The third control animal, AO-409, died on day 30 following a chronic high-level parasitemia with a maximum parasite count of 5.9%.

Two of the five animals immunized with the 3' repeat region (Ag28, group I) were protected. These animals (AO-420 and AO-421) had high antibody levels to the 8-amino acid sequence EENVHDA, the less abundant repetitive sequence in the 3' repeat region, whereas the non-protected animals in this group did not (Table 1). In contrast, all animals in group I, whether or not they were protected, had high levels of antibodies to the 16-amino acid sequence (EENV)₄, four repeats of the major repetitive sequence in the RESA molecule. All animals had positive responses in the IFA and RESA-IFA tests. None of these group I monkeys developed antibodies reactive with the 22-amino acid sequence (DDEHVEEPTVA)₂ from the 5' repeat region.

All four animals in group II (immunized with Ag632, containing the 5' repeat sequences) were protected. All animals had high antibody responses to the 22-amino acid sequence from the 5' repeat region and no detectable responses to the two 3' repetitive sequences. The IFA responses were positive for all animals but were lower than those obtained with group I animals. No positive responses were obtained in the RESA-IFA test.

Three of the five animals in group III (Ag631 + Ag633) were protected. One of the non-protected monkeys (AO-425) died before being treated with a maximum parasitemia of 7.5%. The protected monkeys (AO-416, AO-417 and AO-428) all had high

Table 1 Parasitological and serological responses in animals immunized with fused polypeptides corresponding to different regions of RESA

Animal (karyotype)	Antigen	Maximum parasitemia		IFA	Serological responses (day of challenge)*			
		RBCs infected (%)	Days after challenge		RESA-IFA	ELISA-1	ELISA-2	ELISA-3
Group I								
AO-421(X)	Ag28	0.4	19	320	3,200	<0.1	2.0	1.9
AO-420(XI)		1.9	15	320	6,400	<0.1	1.5	1.8
AO-423(X)		10.2†	11	640	3,200	<0.1	<0.1	1.9
AO-424(V)		16.0†	14	320	400	<0.1	<0.1	2.1
AO-430(X)		19.0†	14	80	400	<0.1	0.2	1.8
Group II								
AO-426(V)	Ag632	1.7	19	40	<100	1.8	<0.1	<0.1
AO-412(X)		1.8	30	160	<100	1.9	<0.1	0.1
AO-427(X)		2.2	27	40	<100	2.0	<0.1	<0.1
AO-422(V)		3.7	19	320	<100	1.9	<0.1	<0.1
Group III								
AO-417(X)	Ag631	0.1	28	40	<100	1.8	<0.1	<0.1
AO-416(X)		+	2.8	16	20	<100	1.4	<0.1
AO-428(V)	Ag633	3.6	11	<10	<100	1.9	<0.1	<0.1
AO-425(X)		7.5‡	20	10	<100	<0.1	<0.1	<0.1
AO-407(XI)		10.2†	14	10	<100	0.2	<0.1	<0.1
Group IV								
AO-409(V)	Control	5.9‡	30	<10	<100	<0.1	<0.1	<0.1
AO-411(X)		11.5†	10	<10	<100	<0.1	<0.1	<0.1
AO-419(XI)		13.0†	11	<10	<100	<0.1	<0.1	<0.1

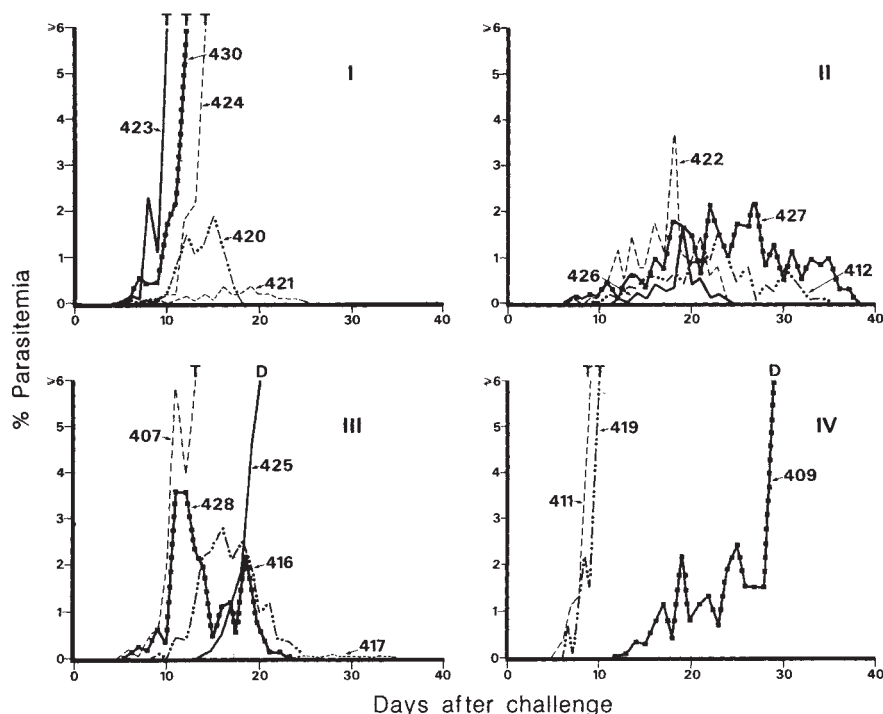
* IFA expressed as reciprocal of the serum dilution end-point using air-dried infected erythrocytes; RESA-IFA, reciprocal of the serum dilution end-point using glutaraldehyde-fixed, air-dried, infected erythrocytes. RBCs, red blood cells. ELISA-1, absorbance value using 22-amino-acid peptide from the 5' repeat region; ELISA-2, absorbance value using 8-amino-acid peptide from the 3' repeat region; ELISA-3, absorbance value using 16-amino-acid peptide from the 3' repeat region. Conventional microELISAs were performed in flexible polyvinyl chloride microtitre plates coated overnight at 4 °C with peptides conjugated to bovine serum albumin (BSA), 5 µg ml⁻¹. Peptide (2 mg) was conjugated to 4 mg BSA with glutaraldehyde. Sera diluted 1:2,000 in 5% non-fat dried milk in phosphate-buffered saline, pH 7.3 were assayed in triplicate against each of the peptide-BSA conjugates. After a 4-h incubation at room temperature, the plates were washed and affinity purified rabbit anti-*Aotus* immunoglobulin conjugated to horseradish peroxidase added for a further 2 h. The plates were again washed and the substrate consisting of 2,2'-azinobis(3-ethylbenzthiazoline sulphonic acid) (Sigma) in 0.1 M citric acid (pH 4.2) containing 0.03% H₂O₂ solution. After 1 h the absorbance of the wells was measured at dual wavelengths (414 nm with reference wavelength 492 nm) using a Titertek Multiscan ML (Flow Laboratories, Finland).

† Animals treated with quinine and mefloquine.

‡ Animals died before treatment.

Fig. 2 Parasitemias in *Aotus* monkeys immunized with recombinant polypeptides of RESA of *P. falciparum*. Group I received Ag28 (3' repeat region of RESA); group II Ag632 (5' repeat); group III Ag631 + Ag633 (non-repeat sequences on either side of the 5' repeat but also including the 5' repeat); group IV (control) TA₁₀ (a fused protein containing uncharacterized sequences of a Taeniid protein). T, animals treated with quinine and mefloquine; D, animals died before treatment.

Methods. Recombinant antigens (250 µg) were emulsified with equal volumes of Freund's complete adjuvant (FCA) and administered in equal doses into the right and left thigh muscles on day -56. Six weeks later (day -14), identical amounts of antigen were emulsified with an equal volume of a 1:10 mixture of FCA and Freund's incomplete adjuvant and administered subcutaneously in two sites over the lower lumbar region of the backs of the monkeys. Final volume for each immunization was 1.5 ml. Each animal was challenged by intravenous inoculation with 1 × 10⁶ blood-stage parasites of the Indochina I/CDC strain of *P. falciparum*.



antibody responses to the 5' repeat 22-amino acid sequence, whereas the two unprotected animals had low levels of antibody to this sequence. No detectable antibody responses to the 3' repetitive sequences were obtained in these animals. IFA responses were minimally positive, and the RESA-IFA responses were negative for all group III animals.

The serological results demonstrate a direct association between protection by immunization with fragments of the RESA molecule and the level of antibody response induced to epitopes encoded by either of two repetitive sequences in RESA. A high-level response to the 11-amino acid sequence in the 5' repeat region was observed in all protected animals in groups II and III. In addition, the two animals from group I that developed high-level responses to the 8-amino acid sequence in the 3' repeat region were protected. The latter result is consistent with the findings of Berzins *et al.*⁷, who reported that rabbit and human antibodies to the RESA 8-amino acid repetitive sequence inhibit merozoite invasion. Most natural, induced antibodies to RESA in humans are directed against epitopes encoded by repeats and these are dominated by specificities reactive with the 4-amino acid repetitive sequence in the 3' repeat (unpublished data). In the present trial, antibodies of this specificity were not associated with protection, and it therefore seems unlikely that high levels of anti-RESA antibodies *per se* will

correlate with immunity (see ref. 4). Further immunization trials with the synthetic peptides used in the serological analysis reported here should determine the potential of the repetitive sequences in RESA as vaccine components.

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Two antigen-independent adhesion pathways used by human cytotoxic T-cell clones

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Cell-cell adhesion is essential for many immunological functions¹⁻⁴, including interaction of cytotoxic T lymphocytes (CTLs) with their targets⁵⁻⁸. We have explored CTL-target interactions using well-characterized cloned human CTLs^{9,10}. Conjugate formation between these CTLs and many antigen-negative targets is almost as efficient as with specific target cells, but does not lead to target-cell lysis. Thus, on specific target cells, adhesion by antigen-independent pathways may occur concurrently with or precede antigen recognition. The molecules LFA-1, CD2 (T11, LFA-2) and LFA-3 have been shown¹¹⁻¹⁵ to be involved in human CTL conjugation with and lysis of specific target cells. Here we describe monoclonal antibody inhibition studies using individual monoclonal antibodies and mixes which demonstrate (1) that LFA-1, CD2 and LFA-3 are involved in antigen-independent conjugate formation; and (2) suggest that CD2 and LFA-3 are involved in one pathway and LFA-1 in another. We confirmed the existence of distinct pathways by the demonstration that LFA-1-dependent adhesion requires divalent cations and is temperature-sensitive whereas CD2- and LFA-3-dependent adhesion does not require divalent cations and is temperature-insensitive. Together with previous data, our studies suggest that CD2 on the effector interacts with LFA-3 as its ligand on targets.

We have analysed the specificity of conjugate formation and of cell-mediated lysis (CML) for a panel of CTLs specific for an allogeneic HLA class II molecule¹⁶ (HLA-DPw2). The antigen specificity of target-cell lysis is shown in Table 1 for a typical CTL clone (designated 8.9); target cells which express the DPw2 allele are lysed and DPw2-negative targets are not. We measured

conjugate formation between CTL and targets by a two-colour fluorescence technique that exploits flow microfluorometry to enumerate conjugates objectively^{17,18}. In contrast to target lysis, the ability of this clone to form conjugates is largely independent of whether or not the target expresses DPw2. Antigen-independent conjugate formation is observed with all DPw2-negative lymphoblastoid B-cell lines (LCLs) tested; conjugate formation is comparable on LCLs from normal donors and LCLs derived from patients with a defect in LFA-1 expression¹⁹, demonstrating that LFA-1 expression on the target is not required for conjugate formation.

Antigen-independent conjugate formation is also observed in various other cell lines; for example, high conjugate formation on U266 (a plasmacytoid cell line grown spontaneously from cells of a patient with multiple myeloma²⁰) and on class II-negative cells such as the cervical carcinoma line HeLa. Conjugate formation also occurs between CTLs and subpopulations of peripheral blood mononuclear cells such as plastic adherent cells or non-adherent non-T cells (E-). Little conjugate formation is seen with resting T (E+) cells as targets. Lysis by this CTL is dependent on target expression of DPw2 but conjugate formation is largely independent of that expression (Table 1).

Antigen-independent conjugates are not artefacts of long-term *in vitro* propagation as they are observed not only with the five clones tested here but also with short-term alloantigen-stimulated T cells²¹ (data not shown). The finding that conjugate formation can occur without target expression of specific antigen and even in the absence of class II expression suggests that, on antigen-positive targets, adhesion precedes or occurs simultaneously with antigen recognition. Defects in this interaction between cells of different species²² may contribute to the inability of many T-cell clones to recognize their specific alloantigen expressed in xenogeneic cells^{23,24}.

Blocking studies with monoclonal antibodies (MAbs) known to inhibit CML demonstrated that α LFA-1, α CD2 and α LFA-3 consistently inhibit antigen-independent conjugate formation (Fig. 1). Potential for antibody-mediated crosslinking between effector and target was minimized by choice of a target (U266) with minimal LFA-1 expression and no CD2; crosslinking by α LFA-3 is not expected as the effectors express only low levels of LFA-3 (data not shown). Blocking by optimal concentrations of these MAbs typically ranges between 20 and 70% and is often less effective at increased antibody concentrations. Previously, antigen-independent conjugate formation has often

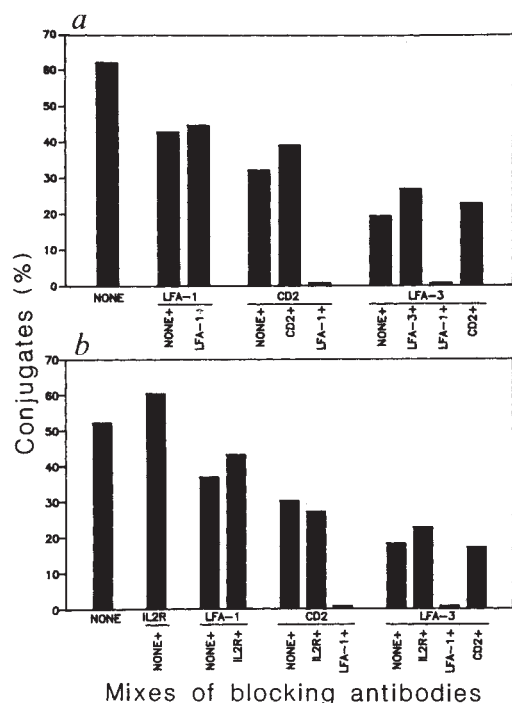


Fig. 1 Antibody inhibition of antigen-independent conjugate formation of two different DPw2-specific CTL clones (8.9 in *a*; 8.2 in *b*) with target U266. MAb was added to the effectors for a 20-min pre-incubation at 37 °C and then remained during the assay of conjugate formation (performed as described in Table 1). The α LFA-1 antibody was MHM23 (refs 13, 34) which is specific for a determinant (also termed CDw18) on the β -chain shared by LFA-1, Mac-1 and p150/95; the α CD2 antibody was 95-5-49 (R.Q. and R.E.G., manuscript in preparation); the α LFA-3 antibody was TS2/9 (refs 11, 12); and the negative control antibody was 7G7/B6 which is specific for the interleukin-2 receptor³⁵. Antibody concentrations were based on results of earlier experiments to give optimal inhibition and were a 1/100 final concentration of ascites fluid for MHM23, TS2/9 and 7G7/B6 and 1/200 ascites for 95-5-49. Each antibody was present at that same concentration in mixes with other antibodies. Results for mixes are shown next to the results for the antibody which was most inhibitory by itself.

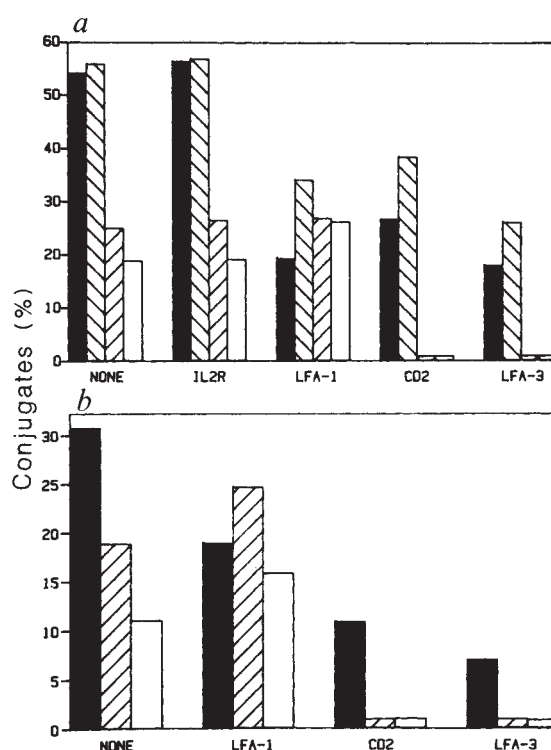


Fig. 2 Differences between pathways of conjugate formation in their requirements for divalent cations and their ability to support conjugate formation at 4 °C. Conjugates were formed between two DPw2-specific clones on two different DPw2-negative targets (clone 8.2 on target U266 in *a*; clone 8.9 on a normal LCLs in *b*). Conjugate formation was measured in the presence of various MABs whose specificity is indicated below the relevant group of bars; antibody concentrations and incubation conditions were identical to those described in Fig. 1 legend. Chelation of divalent cations was achieved by pre-incubation of both effector and target for 20 min at 37 °C with: ■, no inhibitor in the presence of 1.8 mM Mg^{2+} and 1.3 mM Ca^{2+} ; ▨, ▩, in the presence of an excess (5 mM) EDTA or EGTA, respectively; □, 4 °C with no inhibitor. Conjugate formation was measured following pelleting, incubation for 6 min at 37 °C (standard conditions) or for 20 min at 4 °C and resuspension.

been ignored on the assumption that it represents an unexplained *in vitro* artefact. But, the probable physiological relevance of the phenomenon is emphasized by our present finding that the molecules involved in antigen-independent conjugate formation are precisely those which are involved in conjugate formation with antigen-positive targets¹⁵ and which are critical in the lytic interaction as a whole¹¹⁻¹⁴. Recent studies by Spits and co-workers²⁵ support our hypothesis of a critical role of antigen-independent conjugate formation in CML.

When mixes of these MABs are assayed for inhibitory capacity, additive (or even synergistic) effects are observed with a combination of α LFA-1 and either α CD2 or α LFA-3, but the mix of α CD2 and α LFA-3 is no more inhibitory than α LFA-3 alone. One previous study¹¹ found a similar pattern of additivity among these MABs in inhibition of CML. These results are consistent with the hypothesis that the CD2 and LFA-3 molecules are involved in one binding pathway whereas the LFA-1 molecule is involved in a distinct pathway. Our data (Fig. 1) indicate that there are no additional pathways in this model system because the mixture of α LFA-1 and α CD2 or α LFA-3 inhibits completely; with these effectors, no role has been demonstrated for the CD4 molecule in physical conjugate formation (S.S. and G.E.G.L., unpublished observations). If CD2 and LFA-3 participate in the same pathway, then antibodies against both would not have additive effects at optimal concentrations because either alone would block the postulated

CD2/LFA-3 pathway. In contrast, α CD2 or α LFA-3 have enhanced effectiveness when mixed with α LFA-1, because each antibody in the mix is inhibiting a different pathway.

Studies of other agents known to inhibit CML show that some manipulations (chilling to 4 °C and removal of divalent cations) may partially inhibit antigen-independent conjugate formation (Fig. 2); this suggests that only part of the process of conjugate formation is temperature-sensitive or divalent cation-dependent. When conjugate formation is analysed using these agents in combination with MAB blocking, the data confirm this hypothesis. LFA-1-mediated conjugate formation (which remains after α CD2 or α LFA-3 inhibition) is temperature-sensitive and depends on a cation, probably magnesium, which is bound by EDTA but not EGTA. CD2- and LFA-3-dependent conjugate formation (which remains after α LFA-1 inhibition) is temperature-insensitive and divalent cation-independent. The cation-dependence of the LFA-1 pathway is consistent with evidence that magnesium rather than calcium is required for conjugate formation²⁶.

It has previously been shown¹¹ that the inhibitory effect of both α LFA-1 and α CD2 is exerted by binding to the effector cell, whereas the inhibitory effect of α LFA-3 is mediated by binding to the target cell. Our present data suggest that LFA-3 is the ligand for CD2. Additional supporting evidence comes from studies by Wolf and co-workers²⁷ demonstrating that the binding of thymocytes to cultured thymic epithelium is inhibited

Table 1 Lysing of DPw2+ targets and antigen-independent conjugation by clone 8.9

Target cell type	Lysis (%)		Conjugates (%)	
	DPw2+	DPw2-	DPw2+	DPw2-
Experiment 1				
Lymphoblastoid B cell lines				
from normal donors	29	2	54	36
from LFA-1 ⁻ patients	26	1	45	34
Experiment 2				
U266, Plasmacytoid cell line				
HeLa, Class II ⁻ cervical carcinoma	—	1	—	65
Peripheral leukocyte populations	—	2	—	49
plastic adherent	32	3	59	49
non-adherent E ⁻	33	1	31	21
non-adherent E ⁺	5	0	8	4

Results shown are for individual targets but are representative of results with other clones and targets. Cytotoxic T-cell clones were propagated and assayed for CML by ⁵¹Cr-release as described elsewhere⁹. Conjugate formation was measured following pelleting of effector and target together at a ratio of 4:1, incubation for 6 min at 37°C and resuspension in cold phosphate buffered saline. Conjugates were enumerated by a flow microfluorometric assay: effectors were stained to fluoresce green, targets to fluoresce red and conjugates detected as particles that fluoresce at both wavelengths¹⁷; 10,000 particles were analysed and the results expressed as the percentage of targets found in conjugates. We also determined the background caused by coincident detection of red and green cells in a cell mix lacking conjugates (that is, an effector/target mix analysed immediately after mixing); this background ranges from 2 to 13% and is subtracted from experimental values. Target cells were prepared as follows: donor cells were typed for presence of DPw2 as described previously³²; lymphoblastoid B cell lines were standard Epstein-Barr virus-transformed lines derived from local normal donors or from patients with <1% of normal LFA-1 expression¹⁹; U266 is a plasmacytoid cell line²⁰ provided by Dr S. Korsmeyer; mononuclear cells were prepared by density separation of fresh peripheral blood, fractionated by adherence to plastic and then the nonadherent cells fractionated by rosetting with 2-aminoethyl-isothiouonium bromide hydrobromide-treated sheep red blood cells³³.

by α CD2 binding to the thymocyte or α LFA-3 binding to the thymic epithelium. Two pieces of evidence conflict with the hypothesis that CD2 binds LFA-3 (1) high conjugate formation is not observed between CTL and many cell types that express LFA-3 (data not shown), but interaction between CD2 and LFA-3 may depend on post-translational modification of the LFA-3 molecule, as has been dramatically demonstrated for the neural cell adhesion molecule, N-CAM²⁸. (2) Trypsin pre-treatment of target LCL eliminates binding by the α LFA-3 MAb, but cytotoxic interactions still occur and are still blocked by α CD2 (ref. 29). This can be explained if the α LFA-3 antibody binds to a trypsin-sensitive epitope different from the binding site for CD2 and prevents interaction of LFA-3 with CD2 either by steric hindrance or by an allosteric effect.

What is the ligand for LFA-1? It is apparent that homotypic interaction between LFA-1 molecules on the effector and target is not critical. This has been demonstrated by previous studies^{30,31}, and is evident from the present studies in which conjugate formation is as good with LFA-1-deficient LCL as with LFA-1-sufficient LCL (Table 1).

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Note added in proof: Recent studies³⁶ suggest that I-CAM-1 may be an LFA-1 ligand.

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Substrate phosphoprotein availability regulates eclosion hormone sensitivity in an insect CNS

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The final step in the moulting of all insects is ecdysis, the shedding of the cuticle of the previous instar, which is triggered in Lepidoptera by the neurosecretory peptide eclosion hormone¹. This hormone acts directly on the nervous system to release the appropriate motor patterns for larval, pupal and adult ecdyses²⁻⁵, but there are only brief periods near the end of each moult when the nervous system is competent to respond to the hormone. Previous experiments have shown that the action of eclosion hormone on the nervous system at pupal ecdysis in the tobacco hornworm, *Manduca sexta*, is mediated by the second messenger cyclic GMP¹. Here we report that the hormone-stimulated increase in cGMP results in the phosphorylation of two proteins, each with an apparent relative molecular mass (M_r) of 54,000. Moreover, the brief periods during which the central nervous system (CNS) is responsive to eclosion hormone seem to result from the transient presence of these substrate proteins within the nervous system. This provides a novel mechanism by which hormonal responsiveness can be regulated.

Greengard⁶ suggested that cyclic nucleotides exert their effects in the nervous system by stimulating the phosphorylation of specific substrate proteins. In the case of eclosion hormone, the earliest known responses of insect muscle and nerve target

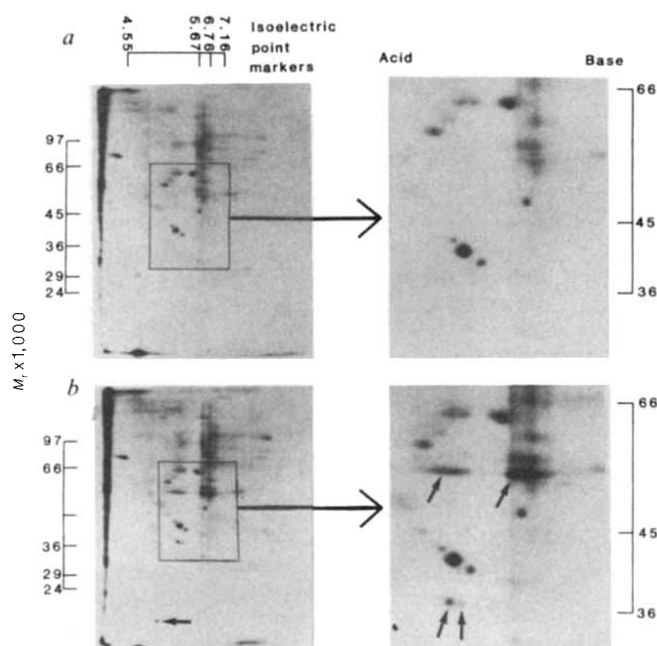


Fig. 1 Phosphorylation of endogenous proteins in the abdominal nerve cord of *Manduca*. *a*, Incubations in the absence of cGMP; *b*, in the presence of 10 μ M cGMP. The enlargement of the central portion of each gel is shown in the right-hand panel. Arrows indicate some of the proteins whose phosphorylation is stimulated by cGMP. Abdominal nerve cords (ANCs) from animals 3.5 h before pupal ecdysis⁸ were dissected and homogenized in 50 mM HEPES buffer, 5 mM EDTA, 1 mM dithiothreitol, pH 7.0 at a concentration of 1 ANC per 150 μ l buffer and centrifuged at 10,000g for 2 min at 4 °C. Phosphorylation was carried out by incubating 100 μ l of the supernatant with 40 μ l 250 mM HEPES buffer, pH 7.0; 20 μ l 100 mM $MgCl_2$; 20 μ l 0.1 mM cGMP or water and 20 μ l 10^{-5} M ATP containing 10 μ Ci [γ - 32 P]ATP (NEN; $\sim 3,000$ Ci mmol⁻¹). The reaction was carried out at 30 °C for 5 min and started by the addition of the ATP and stopped with 200 μ l 20% trichloroacetic acid and allowed to stand on ice for 30 min, centrifuged at 10,000g for 5 min at 4 °C. The pellet was dissolved in 40 μ l 9.5 M urea, 2% Nonidet P-40 (Sigma), 1.6% Ampholines pH 5-7, 0.4% Ampholines pH 3.5-10 (both LKB), 5% 2-mercaptoethanol and 0.01% SDS. The first dimension of separation was by non-equilibrium pH gradient electrophoresis (NEPHGE)⁹, with gels containing 9.2 M urea; 4% acrylamide; 2% Nonidet P-40 and 2% Ampholines pH 3.5-10 (LKB). Samples of 40 μ l (~ 80 μ g protein) were loaded onto the gels and run for 6 h at 500 V, removed from the tubes and equilibrated in 62.5 mM Tris-HCl, pH 6.8, 2.3% SDS, 10% glycerol and 5% 2-mercaptoethanol. The second dimension was separated using SDS-PAGE¹⁰ on 10% acrylamide gels containing 0.1% SDS, run at 50 V for 1 h, then 150 V until the tracking dye was 1 cm from the bottom of the gel. The gels were then stained with Coomassie blue, dried and exposed to preflashed Kodak XRP-1 film with DuPont Lightning Plus intensification screens. M_r standards used were phosphorylase *b* (97,400), bovine albumin (66,000), egg albumin (45,000), glyceraldehyde-3-phosphate dehydrogenase (36,000), carbonic anhydrase (29,000) and trypsinogen (24,000). Isoelectric point markers used were trypsin inhibitor (4.55), human carbonic anhydrase *b* (6.57) and myoglobin (6.76 and 7.16).

tissues is an increase in cGMP^{4,6,7}. To understand how this increase mediates pupal ecdysis, we examined the proteins that are phosphorylated in *Manduca* nerve-cord homogenates in response to cGMP. Addition of cGMP to these cell-free homogenates stimulates the phosphorylation of several proteins (Fig. 1, arrow), the most prominent of which appear as two broad spots, each made up of two or more discrete spots. The two broad spots each have an apparent $M_r \sim 54,000$ (54K) and isoelectric points of 5.35-5.85 and of 6.45-6.75.

We used the back-phosphorylation technique of Forn and Greengard¹¹ to obtain evidence that the 54K proteins are invol-

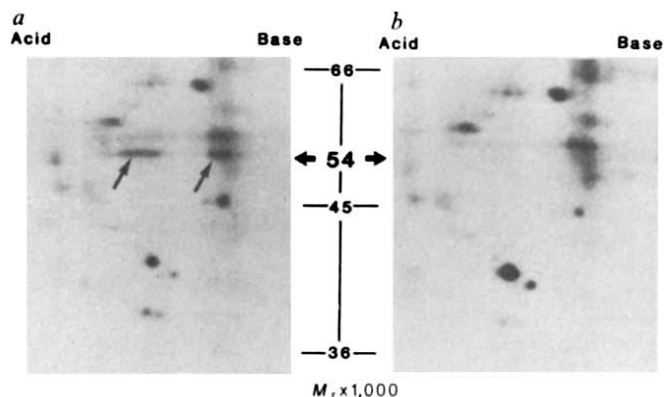


Fig. 2 Back-phosphorylation experiment showing the action of eclosion hormone on phosphorylation of the 54K proteins. *a*, ANC removed 1.5 h after saline injection; *b*, ANC removed 1.5 h after hormone injection. Arrows indicate position of the 54K proteins. Animals at 3.5 h before pupal ecdysis were injected with 0.5 U of hormone or saline. The hormone was partially purified from corpora cardiaca-corpora allata complexes that had been chromatographed through a reverse-phase C-18 column using HPLC. At the onset of ecdysis (1.5 h after injection) the ANCs were removed, homogenized, phosphorylated in the presence of 10 μ M cGMP and separated by two-dimensional SDS-PAGE as described in Fig. 1. Only the portion of each fluorogram shown enlarged in Fig. 1 is shown.

ved in the action of eclosion hormone. We injected prepupae with the hormone or saline, and about 90 min later, at the time the peptide-treated animals were showing induced ecdysis, we removed the ventral nervous systems from both groups of insects, homogenized them and subjected them to *in vitro* phosphorylation in the presence of cGMP. If the 54K proteins are phosphorylated *in vivo* in response to eclosion hormone the appropriate sites on these proteins should be occupied by endogenous, unlabelled phosphate and would be expected to be unavailable for accepting labelled phosphate during the *in vitro* reactions. Figure 2 shows that extracts of the ventral CNS from hormone-treated animals no longer show incorporation of labelled phosphate into the 54K proteins whereas solvent-treated controls have the normal pattern of phosphorylation. Four repeats of this experiment show the complete loss of labelled phosphate incorporation into the 54K proteins after exposure to eclosion hormone. The 54K proteins are the only proteins which reproducibly show this change in response to exposure to the hormone.

Exposure of target tissues to eclosion hormone results in an increase in the endogenous levels of cGMP but not cAMP⁴. But in cell-free homogenates, both cGMP (Fig. 1) and cAMP (data not shown) stimulated the phosphorylation of the two 54K proteins. This lack of specificity is mirrored in intact animals, where the 8-bromo (8-Br-) analogue of both of the cyclic nucleotides mimics the action of eclosion hormone by causing premature ecdysis, with 8-Br-cGMP being about 2.5 times more effective than 8-Br-cAMP⁴. Back-phosphorylation studies indicate that effective dosages of both cyclic nucleotide analogues also result in the *in vivo* phosphorylation of the 54K proteins.

Behavioural sensitivity to eclosion hormone is completely lost during the intermoult period and only appears as the subsequent moult is nearing completion^{8,12,13}. During the moult to the pupal stage in *Manduca*, behavioural responsiveness first appears ~ 8 h before ecdysis and then is lost after the behaviour is performed⁸. In exploring the mechanism by which this responsiveness is regulated, we have shown that the ability of eclosion hormone to cause increases in cGMP is switched on and off during each moult⁴. However, the ability of the CNS to respond to the hormone by increasing endogenous levels of cGMP is established at least 16 h before the CNS is competent to respond

Table 1 Activities of partially purified cyclic nucleotide-dependent protein kinases in the nervous system of *Manduca* during the last 24 h of the pupal moult

Kinase	Addition	-24 h CNS		-3.5 h CNS	
		Activity	Increase (%)	Activity	Increase (%)
cAMP-dependent	Buffer	474 ± 2	—	428 ± 28	—
	0.1 µM cAMP	590 ± 22	24	546 ± 13	28
	0.1 µM cGMP	560 ± 21	18	470 ± 14	10
cGMP-dependent	Buffer	53 ± 5	—	52 ± 2	—
	0.1 µM cAMP	64 ± 5	21	50 ± 3	-4
	0.1 µM cGMP	75 ± 6	42	79 ± 7	52

Values in pmol phosphate transferred per min per mg protein at 30 °C. Mean ± s.e.m. ($n = 4$).

behaviourally to the peptide⁴. A similar relationship is seen in another moth species as certain skeletal muscles acquire the capacity to degenerate in response to eclosion hormone⁷. This difference in timing suggests that the biochemical event(s) controlling behavioural responsiveness is at a stage distal to the hormone-stimulated cGMP accumulation. Likely candidates for this controlling step are the cGMP-dependent protein kinase and the protein substrates that are phosphorylated by this enzyme.

The timing of ecdysis at the pupal moult in *Manduca* can be accurately predicted by external morphological markers⁸. At 22 °C the first signs of pigmentation of the underlying pupal cuticle (the 'trace bars' stage) can be seen at 24 h before ecdysis. At 3.5 h before ecdysis the partially digested larval cuticle becomes folded and creased (the 'anterior shrink' stage). At -24 h, eclosion hormone elevates cGMP levels in the CNS but does not trigger ecdysis behaviour, whereas at -3.5 h, the hormone both elevates cGMP levels and triggers ecdysis⁴.

We assayed the activities of both the cAMP- and the cGMP-dependent protein kinases in the CNS at both of these developmental stages. Partially purified kinases were prepared and assayed according to previously published methods^{14,15}. The assay measured the incorporation of ³²P from ATP into histone H2B (Sigma, type VII) in the presence or absence of 0.1 µM cAMP or 0.1 µM cGMP (Table 1). A Mann-Whitney *U* test showed that there is no significant increase in the activity of the either cAMP- or cGMP-dependent protein kinase between 24 and 3.5 h before ecdysis. Consequently, neither of these enzymes appears to be involved in determining the onset of responsiveness to eclosion hormone.

Because the cyclic nucleotide-dependent protein kinase levels appear not to change during this critical period, we next looked at the 54K proteins. Nervous systems from behaviourally unresponsive animals (-24 h) show no incorporation of labelled phosphate into the 54K proteins, whereas nervous systems from behaviourally responsive animals (-3.5 h) showed the expected labelling of the 54K proteins (Fig. 3A). Further evidence that this difference is due to differences in the substrate proteins rather than protein kinase levels comes from ultracentrifugation experiments. Both of the 54K proteins are pelleted by centrifugation at 100,000g for 1 h, whereas the cGMP-dependent protein kinase remains in the soluble fraction of homogenates¹⁶. We prepared high-speed supernatants and pellets from the CNS from -24- and -3.5-h animals. The supernatants and pellets were interchanged, rehomogenized and the phosphorylation reaction carried out in the presence of cGMP. The reaction mixture was then recentrifuged at 100,000g for 1 h and all the fractions analysed by two-dimensional SDS-polyacrylamide gel electrophoresis (PAGE) and fluorography. The pellet fraction from -3.5-h animals show the presence of labelled 54K proteins irrespective of whether it was incubated with the supernatant from nervous systems from -3.5- or -24-h animals. By contrast, no incorporation of labelled phosphate was seen in the 54K region in pellets from -24-h animals incubated with supernatants from nervous systems from either -3.5- or -24-h animals.

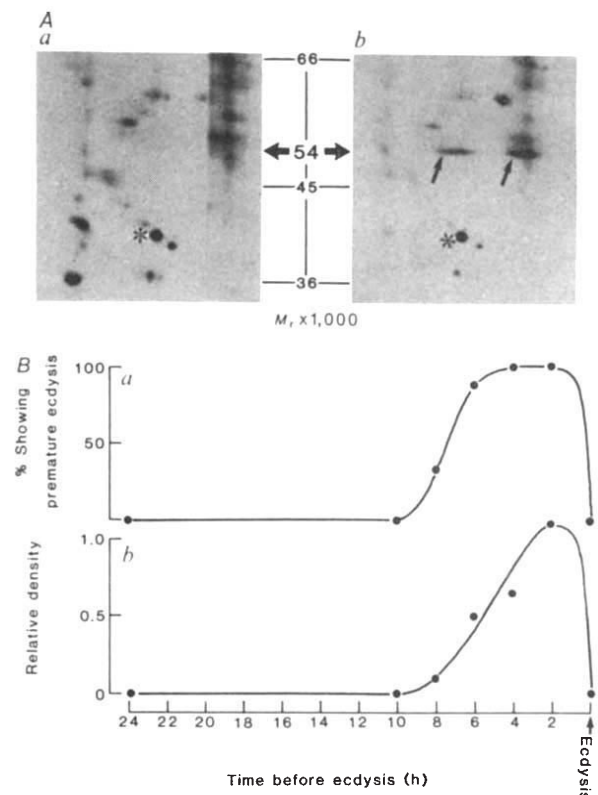


Fig. 3 A, Phosphorylation of endogenous proteins in the ANC at 24 h and at 3.5 h before ecdysis in the presence of cGMP. Proteins were phosphorylated and separated as described previously and only part of each fluorogram is shown. a, ANC from animals 24 h before ecdysis; b, ANC from animals 3.5 h before ecdysis. Arrows indicate the position of the 54K proteins. B, Relationship of the development of responsiveness to hormone and the appearance of the 54K proteins in the CNS. Animals were selected at the trace bar stage (-24 h) and held at 22 °C for various times before treatment. a, Timing of the ability of the hormone to trigger precocious ecdysis behaviour. Groups of at least 10 staged animals were injected with 0.5 U of hormone at each point and the per cent showing premature ecdysis noted. b, Time course of appearance of the phosphorylation of the 54K proteins. Groups of five ANCs for each time point were pooled, homogenized, phosphorylated in the presence of 10 µM cGMP and separated by two-dimensional SDS-PAGE as described in Fig. 1. The portion of the fluorogram above and below the more acidic of the 54K proteins was scanned with a densitometer (Quick Scan Jr, Helena Laboratories) and the area of the peak (1/2 height width × height) compared to the area of a standard protein (at ~40K, marked with an asterisk) which did not seem to change during this time.

A more detailed time course of the appearance of the 54K proteins is shown in Fig. 3B. There is a close association between the time of appearance of the 54K proteins and the onset of behavioural sensitivity to eclosion hormone. At 10 h before the predicted time of ecdysis none of the animals is behaviourally

sensitive to the hormone and no traces of the 54K proteins are seen on the fluorogram. After 2 h, 33% of the animals respond to eclosion hormone and faint traces of labelled 54K proteins are evident in the pooled samples of nerve cords. Subsequently, more animals become sensitive to the hormone and the density of the 54K spots increases. The density of the 54K spots continues to increase beyond the time that 100% of the insects have become behaviourally responsive to the hormone, which may reflect the fact that the response latency between injection and ecdysis continues to be reduced as the insects approach the normal time of hormone release⁸. At the time of pupal ecdysis the insects become behaviourally refractory to eclosion hormone and the labelled phosphate is no longer incorporated into the 54K proteins. We assume that this lack of incorporation is because the action of endogenous hormone has phosphorylated the 54K proteins with unlabelled phosphate in a manner analogous to the back-phosphorylation experiments described above.

We suggest that the ability to label the 54K proteins at about 8 h before ecdysis reflects their *de novo* synthesis at about this time. It is also possible that the activity of phosphatases regulates the apparent protein changes. The use of cycloheximide to block protein synthesis provided preliminary evidence to support the former hypothesis. Injection of 150 µg cycloheximide 10 h before ecdysis (when the 54K proteins are absent) prevented the subsequent appearance of the proteins and also the onset of behavioural sensitivity to eclosion hormone. Injections of cycloheximide 4 h later did not prevent the appearance of the

54K proteins or the ability of the CNS subsequently to respond to the hormone. Thus, the acquisition of sensitivity to eclosion hormone requires the synthesis of new proteins, probably the 54K proteins.

The evidence presented here demonstrates that the two 54K proteins are phosphorylated, via cGMP, by the action of eclosion hormone on the CNS of *Manduca*. We also suggest that the transient sensitivity shown by the nervous system to the hormone is regulated by the transient presence of these proteins in the CNS.

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Localization of D-2 dopamine receptors to intrinsic striatal neurones by quantitative autoradiography

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Recent work with positron emission and single photon emission computed tomography has demonstrated the feasibility of studying striatal dopamine receptors in the living human brain¹⁻³. For the proper interpretation of these studies in normal and diseased states, the cellular localization of these receptors must be definitively established. It has been claimed, on the basis of receptor binding studies with tissue homogenates in rats, that 30-50% of striatal D-2 dopamine receptors are located on axons or terminals of the corticostriatal pathway⁴⁻⁶. This finding has been incorporated into major reviews and classifications of dopamine receptors^{7,8}. The recent development of quantitative autoradiographic methods for diffusible ligands has facilitated the study of neurotransmitter receptors in cytoarchitecturally intact tissue^{9,10}. Because this technique provides the necessary anatomic resolution that is lacking in homogenate binding studies, we have used it to re-examine the localization of striatal dopamine receptors. Here we present evidence that D-2 receptors are located exclusively on kainic acid-sensitive intrinsic neuronal elements in the striatum. We report that discrete cortical ablation does not alter ³H-spiperone binding to rat striatum and thus our results do not support the existence of D-2 dopamine receptors on the terminals of the corticostriatal pathway.

We made unilateral cortical lesions in ketamine-anaesthetized male Sprague-Dawley rats by suction within a 3×6 mm rectangular burr hole (coordinates: 1-4 mm L, -2 to +4 mm from bregma, AP). We injected the rats prophylactically with Bicillin (300,000 units intramuscularly). We confirmed lesions histologi-

cally and excluded those that extended through the corpus callosum to damage nonspecifically the striatum. The time course and location of degenerating nerve terminals within the striatum were verified using a silver degeneration stain¹¹. At 5-7 days following cortical lesion, degenerating nerve terminals were localized to the dorsal aspect of the neostriatum. Unilateral striatal kainic acid (KA) lesions were made by injecting 0.8 µg 0.4 µl⁻¹ saline, pH 7.0, over 5 min (coordinates: 7.8 A, 2.8 L, -5.5 V)¹². We injected 2.5 mg kg⁻¹ diazepam intraperitoneally to minimize distant neuronal degeneration. Rats were killed 5, 10, 20 and 40 days after cortical or KA lesion and the brains rapidly removed and frozen in liquid freon. Serial 20-µm frozen sections were cut through the striatum for binding studies. Sections were incubated at room temperature for 30 min with 2 nM ³H-spiperone (³H-SPIP) (18 Ci mmol⁻¹; NEN) in 50 mM Tris-acetate, 154 mM NaCl buffer, pH 7.4. Adjacent sections for nonspecific binding were incubated with ³H-SPIP plus 2 µM (+)butaclamol. Alternate sections were incubated with 1 nM ³H-QNB (L-[benzyl-4,4'-³H]-quinuclidinyl benzilate; 38 Ci mmol⁻¹; NEN) at room temperature in phosphate-buffered saline, pH 7.4, with or without 1 µM atropine. Sections were exposed with calibrated ³H standards (³H-Microscales, Amersham) to LKB Ultrofilm. Film autoradiographs were analysed densitometrically with a Leitz MPV variable aperture microdensitometer. For rats with cortical lesions, densitometric measurements were recorded over the dorsal aspect of the striatum, where degenerating nerve terminals had been demonstrated by silver stain. For rats with KA lesions, measurements were taken within the lesion borders. A minimum of four tissue sections were analysed for total binding, and four sections for nonspecific binding, per rat, with a minimum of four rats per time point. Dunnett's multiple *t*-test was used for statistical comparisons.

Correction for tritium autoabsorption was performed according to established methods^{13,14}. A correction factor of 32% was applied to the optical density values from all groups as tritium autoabsorption in the lesioned striata was the same as that from the contralateral intact striata and from controls.

Our results are summarized in Table 1 and Fig. 1. At 5, 10 and 20 days after discrete cortical lesion, specific binding of

Table 1 Effects of cortical ablation and striatal kainic acid lesion on ^3H -SPIP and ^3H -QNB binding to rat striatum

Cortical lesion		Controls <i>n</i> = 4	5 days <i>n</i> = 4	10 days <i>n</i> = 4	20 days <i>n</i> = 4	40 days
^3H -SPIP	Ipsilateral	214 ± 45	215 ± 16	198 ± 47	187 ± 9	—
	Contralateral	218 ± 35	194 ± 23	216 ± 33	186 ± 21	—
^3H -QNB	Ipsilateral	432 ± 85	526 ± 83	526 ± 118	506 ± 66	—
	Contralateral	435 ± 87	484 ± 93	506 ± 90	477 ± 67	—
Striatal KA lesion		<i>n</i> = 4	<i>n</i> = 4	<i>n</i> = 4	<i>n</i> = 6	<i>n</i> = 8
^3H -SPIP	Ipsilateral	218 ± 35	145 ± 9*	85 ± 9*	55 ± 29*	22 ± 11*
	Contralateral	214 ± 45	185 ± 11	180 ± 33	164 ± 44	169 ± 21
^3H -QNB	Ipsilateral	435 ± 87	426 ± 53	237 ± 70*	139 ± 75*	113 ± 43
	Contralateral	432 ± 85	525 ± 80	422 ± 68	494 ± 98	646 ± 87*

Specific binding of ^3H -SPIP and ^3H -QNB (fmol mg^{-1} tissue wet weight, mean $\pm$ s.d.) to rat striatum at 5, 10, 20 and 40 days after discrete cortical ablation or striatal KA lesion. Specific binding represents 50–60% of total binding for ^3H -SPIP and 95–99% of total binding for ^3H -QNB. The ligand concentrations used in these binding assays exceed K_D values by ~ 10 -fold; therefore, the reported specific binding values closely approximate B_{max} . Dunnett's *t*-test was used to compare the experimental groups to unlesioned controls; *n* = no. of rats per group. Asterisk, $P \leq 0.01$. Binding of ^3H -SPIP and ^3H -QNB is not altered at any time-point after cortical lesion. Binding of both ligands decreases significantly in a time-dependent manner within the boundaries of the KA lesion.

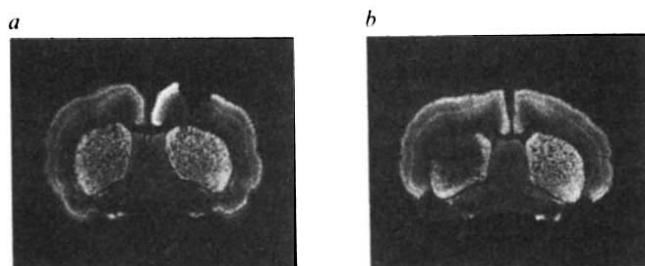


Fig. 1 Reversed-image autoradiographs depicting total ^3H -SPIP binding. White regions, high in binding; dark regions, low in binding. *a*, Five days after cortical lesion, there is no change in ^3H -SPIP binding in the ipsilateral striatum, but there is increased binding in the ipsilateral cingulate cortex that is not displaced by (+)-butaclamol. *b*, ^3H -SPIP binding is markedly reduced 20 days after striatal lesion. Note the rim of spared striatal tissue with normal binding. Adjacent sections incubated with ^3H -QNB (not shown) demonstrated the same degree of binding loss within the lesion boundaries as well as an identical rim of unaffected tissue.

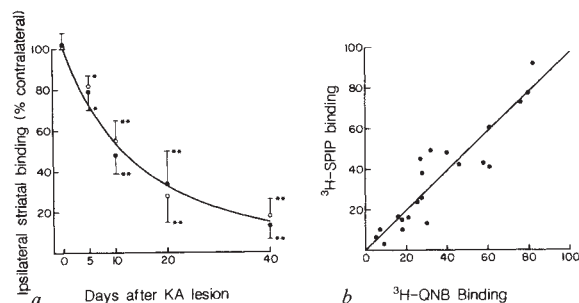


Fig. 2 *a*, Time-course of ^3H -SPIP (●) and ^3H -QNB (○) binding after unilateral striatal KA lesion. Each point represents the percentage of binding within the lesion (mean $\pm$ s.d.) compared with the intact contralateral striatum. Dunnett's *t*-test was used to compare experimental groups with controls. Asterisk $P \leq 0.05$; double asterisk, $P \leq 0.01$. *b*, Correlation between ^3H -SPIP and ^3H -QNB binding (expressed as percentage of contralateral striatum) within the KA lesion. Each point represents data from 1 rat, 5–40 days after lesion. $r = 0.92$, $P < 0.01$, d.f. = 19.

^3H -SPIP and ^3H -QNB in the striatum ipsilateral to the lesion, and in the contralateral striatum, is the same as in unlesioned controls. In contrast, the KA striatal lesion results in a time-dependent decrease in binding for both ligands (Fig. 2). When binding within the lesion is expressed as a percentage of the contralateral side, specific binding of ^3H -SPIP is reduced to 79, 48, 34 and 13% at 5, 10, 20 and 40 days after KA lesion,

respectively. Specific binding of ^3H -QNB is similarly reduced to 82, 55, 28 and 18% at the same time-points. Linear regression analysis reveals a correlation between ^3H -SPIP and ^3H -QNB binding remaining in the lesion for each animal (Fig. 2*b*; $r = 0.92$, $P < 0.01$, d.f. = 19).

The results suggest that D-2 dopamine (DA) receptors are located exclusively on intrinsic neurones within the striatum and not on the terminals of the corticostriatal pathway. When comparing the results of this autoradiographic study with conflicting results from previous tissue homogenate studies, we first considered anatomic specificity. The assumption that the neuronal elements within the KA-lesioned striatum have been totally and homogeneously destroyed is inherent in the interpretation of the homogenate studies. From the autoradiograph in Fig. 1*b* and from detailed morphological studies of striatal KA lesions¹⁵, it is clear that a variable rim of normal striatum surrounds the lesioned tissue, which itself contains a small population of KA-resistant cells. We found, at the level of the body of the striatum, that the KA lesion occupies 60% of the total striatal area, whereas ^3H -SPIP binding in the rim of non-lesioned tissue is equal to control values. If we had assumed that the entire striatum is homogeneously lesioned, and recorded optical densities over the entire striatal area (the autoradiographic equivalent of a homogenate study), we would have underestimated the extent of the binding decrement induced by KA. In published reports using tissue homogenates, where the anatomical extent of the lesion necessarily varies relative to the dissected striatal volume, the mean decrement in ^3H -SPIP binding following striatal KA lesion is $\sim 50\%$, an underestimate of the maximal decrement within the lesion^{4,5,16–23}.

The second factor we considered in interpreting our results is the temporal profile of the binding loss following the KA lesion. The data clearly demonstrate that ^3H -SPIP and ^3H -QNB binding decrease at the same rate and to the same extent from 5 to 40 days following lesion (Fig. 2). At 21 days after KA infusion, disrupted neurones and phagocytic cells can be seen in the light microscope, and electron microscopy reveals intact synaptic complexes within the lesioned tissue^{15,24}. Therefore, the time-course of the binding decrement may reflect the removal of neuronal membrane fragments. At 40 days after KA lesion, ^3H -SPIP binding within the lesion borders is reduced to $13 \pm 6\%$ (mean $\pm$ s.d.) of contralateral values, with a range of 3 to 23%. The residual binding at 40 days may be to synaptic elements which have not yet been cleared or may represent binding to intrinsic striatal neurones not destroyed by the KA. Clearly, ^3H -SPIP binding within a KA lesion can be reduced to $< 5\%$ of control values at 40 days. Previous studies of ^3H -SPIP binding at early time-points (1–4 weeks) following lesion therefore underestimate the extent of the maximal binding decrement^{4,5,17–23}.

We used ^3H -QNB binding as a marker for intrinsic striatal neurones. Our data demonstrate that, following KA, decreases in ^3H -SPIP and ^3H -QNB binding (expressed as per cent of contralateral values) are equivalent (Fig. 2b). The evidence that both the time-course and extent of ^3H -SPIP and ^3H -QNB binding changes following KA are the same, and that binding of both ligands can be reduced to <5% of control values at 40 days after KA lesion, suggests that D-2 DA receptors are located exclusively on KA-sensitive, intrinsic striatal neurones. These results conflict with those of a previous study by Murrin *et al.*²⁵ in which two rats with extensive cortical ablations were studied after the intravenous injection of ^3H -SPIP. Silver grains were counted over a $3,600\ \mu\text{m}^2$ region of the striatum from a total of 7 tissue sections, with a reported 18% decrement in grain density in the striatum ipsilateral to the cortical ablation. The conflicting results may reflect the different methods used. Our conclusions are supported by other homogenate binding studies and by recent autoradiographic studies²⁶⁻²⁹.

The cortical lesion used here was specifically designed to ablate a 3×6 mm region of frontal sensorimotor cortex known to project primarily to the dorsolateral sector of the striatum³⁰. We used this relatively discrete lesion to avoid unintended damage to the underlying striatum, which can complicate more extensive cortical ablation. In the study of Schwarcz *et al.*⁴, extensive cortical ablation resulted in a 27% decrement in striatal choline acetyltransferase activity (an enzyme marker for intrinsic striatal neurones), casting doubt on the significance of the 32% reported reduction in ^3H -haloperidol binding. In our present study, at 5-7 days following cortical lesion, we found many degenerating nerve terminals in the dorsal striatum in a pattern conforming to the reported topographic corticostriatal projection. If, as reported, 30-50% of striatal D-2 DA receptors are located on terminals of corticostriatal axons, their absence following cortical lesion would probably be visible in the autoradiographs. By emphasizing both the anatomical specificity of a lesion and the temporal course of subsequent binding changes, we confirm the use of quantitative autoradiography for receptor localization studies in the central nervous system.

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Single-channel and whole-cell recordings of mitogen-regulated inward currents in human cloned helper T lymphocytes

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Cytoplasmic free Ca^{2+} ($[\text{Ca}^{2+}]_i$) appears to be an important signal for DNA synthesis in early stages of lymphocyte activation. In spite of many experimental studies which employ fluorescent Ca^{2+} indicator dye to demonstrate an early increase of $[\text{Ca}^{2+}]_i$ in T-lymphocytes after stimulation with lectins¹⁻³, specific antigens^{4,5}, and monoclonal antibodies to T-lymphocyte receptors⁶⁻⁸, the mechanism responsible for the rise of $[\text{Ca}^{2+}]_i$ is unknown. We have used the extracellular patch clamp technique⁹ to investigate this mechanism. Unitary inward currents, mediated by Ca^{2+} or Ba^{2+} , were recorded in the membrane of T-lymphocytes. The inward current channel was characterized by a conductance of 7 pS and extrapolated reversal potential (E_{rev}) 110 mV positive to resting potential (V_r). While gating kinetic parameters were not affected by membrane potential changes, the probability of channel opening markedly increased upon activation of the T-lymphocyte by the mitogenic lectin, phytohaemagglutinin (PHA). PHA also evoked a cadmium-sensitive, inward Ba^{2+} current on whole-cell clamp. We suggest that this mitogen-regulated channel introduces Ca^{2+} into the cytoplasm upon activation and represents a new class of voltage-independent Ca^{2+} channels.

We recorded inward currents at V_r from more than 50 different cell-attached patches of human cloned helper T-lymphocytes¹⁰ with pipettes containing various solutions, including normal Ringer solution and tetrodotoxin (TTX)-containing, isotonic Ca^{2+} -tetraethylammonium chloride (TEACl) and Ba^{2+} solutions. Figure 1a shows representative inward currents from a cell-attached patch recorded at V_r with a pipette containing isotonic BaCl_2 . The inward currents, with approximate amplitude of 0.8 pA at this potential, appeared as intermittent bursts of brief openings. This patch typifies the more than 50 patches containing the channel; however the great majority of patches tested contained no inward current activity, suggesting a relatively low channel density.

Figure 1b represents the unitary current-voltage ($I-V$) relation for the inward current channel, recorded with isotonic BaCl_2 pipettes. Measurements made from cells suspended in physiological solutions, with an assumed V_r of -60 mV (ref. 11), as well as from cells in which V_r was zeroed by high external KCl, are represented. An ohmic $I-V$ relation was observed for the range from V_r-40 mV to V_r+100 mV. Similar results were observed in 14 cell-attached patches with an overall conductance range of 4.8 to 9.4 pS (7.4 ± 1.36 , $n = 14$). Since clear E_{rev} could not be obtained for the voltage range tested, E_{rev} were estimated by extrapolation at 109 ± 26.5 mV ($n = 9$) and 78 ± 22.2 mV ($n = 5$) positive to V_r in the cell-attached patches suspended in normal Ringer solution and 150 mM KCl solution, respectively.

While single-channel amplitude was dependent on membrane potential, channel gating behaviour did not appear to be appreciably affected by either depolarizing or hyperpolarizing voltage jumps. Channel activity was present over the entire voltage range studied, and there was no qualitative change in the channel appearance at different potentials. Quantitative analysis was performed to investigate voltage-sensitivity of gating further. Open- and closed-time histograms constructed from a 30-sec

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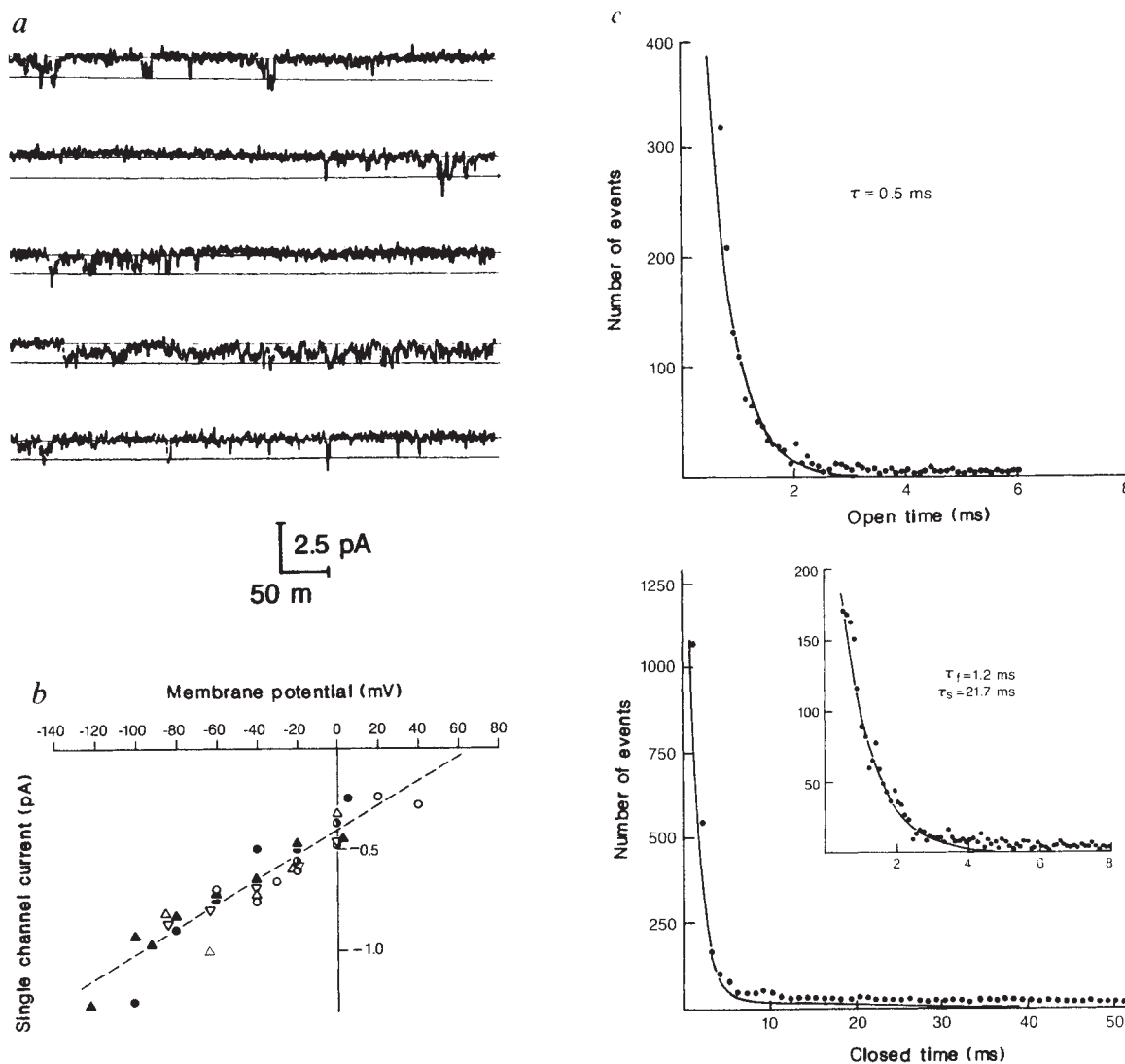


Fig. 1 Characteristics of unitary inward currents recorded in cell-attached configuration with barium pipette solution. *a*, Sample records of unitary inward currents recorded at resting potential from one patch. The patch pipette contained 110 mM BaCl₂, 200 nM TTX, and 10 mM HEPES-KOH (*pH* 7.3). Inward current is seen as a downward deflection from the baseline. The upper line indicates the baseline and the lower line indicates the average unitary current amplitude in each trace. Bandwidth was 1.0 kHz (−3 dB). Sampling rate was 4 kHz. *b*, Unitary current-voltage relationships. The same symbol represents the same patch; the plot represents cumulative results from six different patches. Each point represents the average amplitude of the unitary currents at the corresponding holding potential obtained from 10–30 measurements by eye of long, well-resolved unitary currents (briefer events were excluded because of error introduced by bandwidth attenuation). For closed symbols, the bath contained (in mM): 154 NaCl; 5.5 KCl; 2.0 CaCl₂, 1.0 MgCl₂; 10 HEPES-NaOH (*pH* 7.3). Patch potential is calculated from an assumed resting potential of −60 mV. For open symbols, the bath solution contained (in mM): 150 KCl, 1 CaCl₂, 2 MgCl₂, 1.1 EGTA; 10 HEPES-KOH (*pH* 7.3). In this case, the patch potential was calculated from an assumed resting potential of 0 mV. Linear regression line is the least squares fit of the data. *c*, Distributions of open-times and closed-times between openings for one-cell-attached patch at resting potential. The patch pipette contained: 110 mM BaCl₂, 200 nM TTX, and 10 mM HEPES-KOH (*pH* 7.3). The bath contained: 150 mM KCl, 1.0 mM CaCl₂, 2 mM MgCl₂, 1.1 mM EGTA, and 10 mM HEPES-KOH (*pH* 7.3). Current signals were filtered at 1 kHz (−3 dB). The duration of record analysed was 30 s with a total of 2,441 openings. The open-time and burst duration distributions were fit with one exponential, while the closed-time distribution was fitted with the sum of two exponentials. The inset above the closed-time distribution represents the same data and fitted curve plotted over a time interval of 0.5 to 8 ms. The time constants are indicated above each histogram.

Methods. Records were from human cloned helper T lymphocytes at least 3 days after restimulation by irradiated stimulator cells. Cells were suspended in physiological solution or high KCl solution as noted above. Cell-attached patch-clamp recordings were made at room temperature (20–22 °C). The patch pipette resistance was between 8 and 20 MΩ, and pipette membrane seal resistance was 10–100 GΩ. Filtered records were digitized at 10 kHz, stored on a microcomputer (IBM PC-AT), and analysed by an automated single-channel analysis program (IPROC-2 and NFITS; Drs C. Lingle, Florida State University and F. Sachs, State University of New York at Buffalo), using an event-detection threshold of 0.5 times the predetermined single channel amplitude, with times of transition defined as crossings of the midline between the baseline and open current level. Multiple event exclusion threshold was set at 1.8 times the computer-derived mean single channel amplitude. Patches used for kinetic analysis contained few, if any, multiple events. To exclude false events, filtering was set to reduce baseline noise to less than one-half event detection threshold. Distribution of open-times, closed-times, and burst durations were fit with probability density functions by the method of maximum likelihood to the continuous distribution of data. The first five bins (500 μs) of the open-time and short closed-time distributions were excluded from the curve-fitting because of error of inadequately resolved events secondary to bandwidth attenuation.

current recording of a cell-attached patch at V_r are shown in Fig. 1c. The open-time histogram was described by a monoexponential function with a time constant of 0.5 ms. The closed-time histogram was approximated by the sum of two exponential functions with fast and slow time constants of 1.2 ms and 21.7 ms, respectively, differing by over an order of magnitude. This corresponds to the observation that groups of openings tended to occur in bursts with longer interburst intervals and indicates the existence of at least one open and two closed states for the ion channel. A third long-lived closed state is suggested by the presence of quiescent intervals of several seconds to minutes in some records that tended to group bursts of openings together in clusters. However, the long-lived closed interval occurred at too low a frequency to appear in probability density distributions, often constructed from 30–60 second recordings. Similar kinetic analysis was performed at three widely spaced potentials in the same patch; the cumulative results of gating kinetic parameters at the different potentials are presented in Table 1. It is apparent that gating kinetics of the inward current channels are not voltage-sensitive. This would suggest that if the channels are Ca^{2+} permeable, they manifest fundamentally different gating behaviour from the electrically excitable Ca^{2+} channels of neurones or muscle cells (for example see refs 12, 13). Indeed, no voltage-gated Ca^{2+} channels of a classical nature have been reported in several previous patch clamp studies of T-lymphocytes^{14–18}.

The effects of PHA on the activity of the inward current channel are demonstrated in Fig. 2, recorded with a Ca^{2+} -TEACl pipette at V_r from a cell-attached patch. Before application of PHA, infrequent inward currents were observed. Approximately 120 seconds after addition of $6.5 \mu\text{g ml}^{-1}$ PHA into the bath, inward current activity increased. Similar augmentation of the inward current after bath application of PHA was observed in 12 cell-attached patches, including 3 patches in which no inward currents were observed before PHA application. In 6 cell-attached patches which showed rather vigorous inward currents before application of PHA, augmentation was not observed. The PHA effect on the inward current occurred within 20 seconds to 5 minutes after addition to the bath, and persisted for at least several minutes thereafter. This time course is consistent with the previously published time course of increased cytosolic Ca^{2+} , as determined by fluorescent indicator dyes, following mitogenic stimulation^{1–3}.

Two other points about the PHA effect are noteworthy. First, PHA must exert its effect via an intermediate metabolic event, since formation of the high resistance gigaohm seal precludes the diffusion of PHA, applied in the bath solution, into the area of membrane directly under the recording pipette. Internal free Ca^{2+} ions are not the activating second messengers, however, since an abrupt reversible termination of single-channel activity was invariably seen after isolation of the membrane patch into internal solutions containing $\geq 10^{-6} \text{ M Ca}^{2+}$ (see Fig. 2c). Second, PHA appears to augment inward current through an effect on the channel gating mechanism, as demonstrated in Fig. 2d, which shows the results of analysis of unitary inward currents in continuous records of a cell-attached patch, measured 2 minutes before and 8 minutes after PHA was applied to the cell. While PHA activation did not markedly affect the unitary current amplitude at V_r , per cent open-time increased dramatically after PHA exposure, chiefly due to marked increase in number of openings, from 1,161 in the 2-minute interval prior to PHA application to 10,480 in the final 2-minutes after PHA application.

The whole-cell variation of the patch clamp technique was used to study macroscopic inward currents activated by PHA. Figure 3a demonstrates divalent cation-mediated inward current in whole-cell records obtained under conditions to minimize contamination by other currents (TTX-supplemented isotonic BaCl outside and EGTA-buffered 160 mM CsCl inside). A family of linear currents was seen in all cases studied under

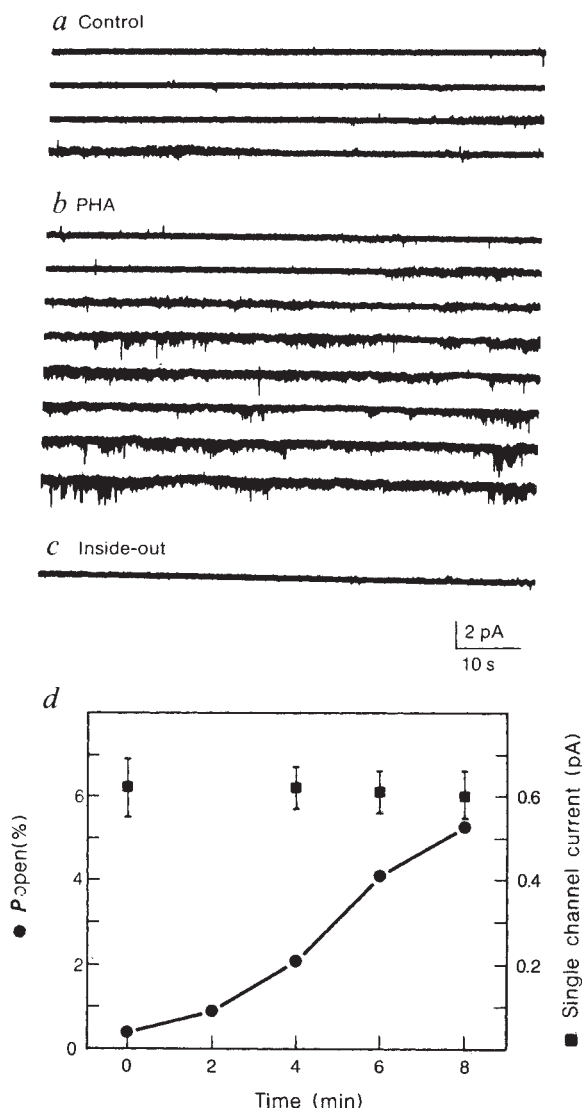


Fig. 2 PHA-induced augmentation of the inward currents. The patch pipette contained: 50 mM CaCl_2 , 60 mM TEACl, 200 nM TTX, and 10 mM HEPES-KOH (pH 7.3). The bath contained: 154 mM NaCl, 5.5 mM KCl, 2 mM CaCl_2 , 1 mM MgCl_2 , and 10 mM HEPES-NaOH (pH 7.3). *a* and *b* are consecutive recordings in a cell-attached patch before (*a*) and (*b*) application of PHA. In *a*, both outward currents (upward deflections) and rare brief inward currents (downward deflections) can be seen. PHA ($6.5 \mu\text{g ml}^{-1}$) was applied at the beginning of the recording *b*. The frequency of the inward current openings increased approximately 120 s after the application, and multiple levels were seen for the first time. *c*, No inward current was observed in the inside-out patch isolated following the recording *b*. The internal surface of the patch faced physiological solution containing 2 mM Ca^{2+} . All recordings were filtered at 1 kHz (-3 dB). Because of bandwidth limitation of the chart recorder, the amplitudes of the rapid currents were slightly smaller than the values obtained with oscilloscope display. *d*, Per cent open-time, (P_{open} , left vertical axis) and single channel current amplitude (right vertical axis) as a function of time after exposure to PHA. Single channel currents were measured in a cell-attached patch at resting potential, as in *a* and *b*. Time interval is represented on the horizontal axis where 0 represents the 2-min interval prior to PHA (1,161 openings); point 2 represents the 2-min interval immediately after PHA (2,576 openings); 4 represents the interval 2–4 min after PHA (4,741 openings); 6 represents the interval 4–6 min after PHA (6,041 openings); 8 represents the interval 6–8 min after PHA (10,480 openings). In total, a consecutive 10-min recording was analysed. P_{open} was calculated as total open-time divided by total time of the record. Mean single channel current amplitude was calculated by eye from 10–20 long, well-resolved events in each record.

Table 1 Effect of membrane potential on single Ca^{2+} channel kinetic parameters from a cell-attached patch.

Membrane potential	0 mV	-40 mV	-84 mV
No. of openings	2,448	2,821	2,321
Open-time (ms)	0.45	0.53	0.56
Closed-time (ms)			
τ_f	1.19	1.15	1.18
τ_s	21.76	11.63	14.51
Burst duration (ms)	3.51	3.62	3.30
No. of events per burst	2.3	2.1	2.1
Per cent open-time	5.97	7.86	6.34
No. of events per second	74.7	86.1	70.8

The patch pipette contained: 110 mM BaCl_2 , 200 nM TTX, and 10 mM HEPES-KOH (pH 7.3). The external solution contained: 150 mM KCl, 1 mM CaCl_2 , 2 mM MgCl_2 , 1.1 mM EGTA, and 10 mM HEPES-KOH (pH 7.3). The total duration of recording was 30 s at each potential. Membrane potentials are expressed relative to resting membrane potential, V_r , with 0 mV equal to V_r . The time constants representing open-time, closed-time, and burst duration, as well as number of events per burst, were obtained from the probability density functions fit by maximum likelihood to the continuous distribution of data, as outlined in Fig. 1 legend. Per cent open-time is calculated as total open-time over total time of record (30 s).

these conditions ($n = 15$), without any apparent voltage- or time-dependent inward current. Changes in holding potential did not alter the inability to elicit inward currents. When the same cell was exposed to PHA (middle trace), a large inward current shift, seen as a downward deflection, occurred at holding potential, as well as all potentials more negative than +20 to +40 mV. This inward current developed gradually, beginning between 30 seconds and 3 minutes after exposure to PHA. The PHA-activated current was abolished upon exposure to the inorganic Cd^{2+} channel blocker Cd^{2+} (lower trace). Cd^{2+} block was immediately reversible upon reperfusion with pure isotonic BaCl_2 (not shown). A cadmium-sensitive outward current component was seen at potentials more positive than +20 to +40 mV ($E_{\text{rev}} = 24 \pm 8$ mV [$n = 5$], calculated from macroscopic $I-V$ relationship of cadmium-sensitive current; see Fig. 3b). This could represent Cd^{2+} -sensitive outward current through another channel or alternatively, monovalent cation (Cs^+) permeation through the mitogen-regulated channel. It has been shown that monovalent cations can carry currents through classical voltage-activated Ca^{2+} channels when the membrane potential is shifted to large positive values or when the divalent cation in external solution is reduced^{12,19-23}. Because of this apparent lack of selectivity, reported E_{rev} for classical Ca^{2+} channels vary from as low as $+26.5 \pm 2.1$ mV ($[\text{Ba}^{2+}]_o = 10$ mM) in neoplastic B

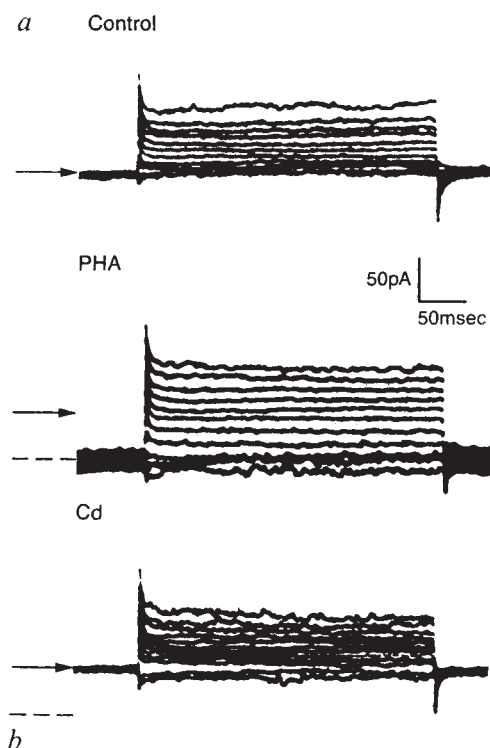
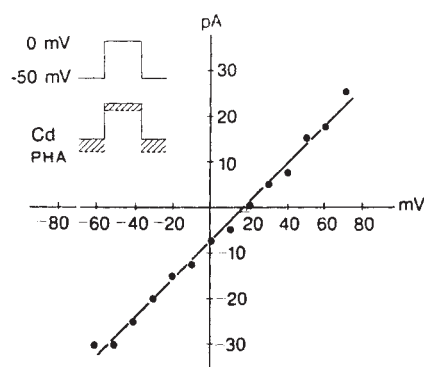


Fig. 3 Whole-cell voltage clamp currents. Cells were suspended in 110 mM BaCl_2 , 10 HEPES-KOH, pH 7.3, with 200 nM TTX. The internal pipette was filled with 160 mM CsCl , 11 mM EGTA, 1 mM CaCl_2 , 5 mM HEPES-NaOH, pH 7.2. *a*, Total membrane currents were recorded during a series of depolarizing and hyperpolarizing pulses from a holding potential of -40 mV to levels spaced 10 mV apart, from -60 to +60 mV. Current signals were filtered at 1 kHz (-3 dB) and photographed from a Tektronix 5111A storage oscilloscope. The three samples were recorded in control high Ba^{2+} solution (Con), 2 min after addition of PHA to the bath (PHA), and immediately after subsequent addition of 5 mM CdCl_2 to the bath (Cd). The arrow indicates the baseline current level in control recordings at holding potential. The dashed line represents the baseline current level at holding potential after PHA application. *b*, Current-voltage relation for cadmium-sensitive linear current component (holding potential equal to -50 mV in case represented here). Inset schematic represents records in a PHA-activated cell obtained before and after Cd^{2+} application and shows how current amplitude was measured. The closed circles represent the Cd -sensitive linear component in the PHA-treated cell. Straight line is the least-squares fit of the data.



lymphocytes²³ to +100 mV ($[Ba]_o = 100$ mM) in reconstituted cardiac calcium channels²⁴.

The present study presents evidence for an inward current with distinct properties in T lymphocytes. Activity of the channel is clearly augmented by exposure of the cell to the mitogenic lectin PHA, suggesting its involvement in the early stage of T-lymphocyte activation. Whether or not this channel is Ca^{2+} -permeable, and thus a mechanism for the introduction of Ca^{2+} into the cytoplasm, is an important question. The mitogen-regulated channel is similar to previously described classical Ca^{2+} channels with respect to its low single channel conductance, positive E_{rev} , permeability to Ba^{2+} ions, a bursting appearance which is described by a single component open-time distribution ($\tau < 1.0$ ms) and two-component closed-time distribution, and reversible antagonism by the inorganic Ca^{2+} channel blocker Cd^{2+} . However, the T-lymphocyte mitogen-regulated channel differs from classical Ca^{2+} channels in two significant ways. First, although it has a positive E_{rev} , whole-cell current records indicate that it deviates even further from the thermodynamic E_{Ca} predicted by the Nernst equation than do most classical calcium channels. This suggests an even lesser degree of monovalent cation discrimination. Second, the mitogen-regulated channel manifests a gating process that is apparently independent of voltage over the range tested. While several recent reports have documented two or more subtypes of Ca^{2+} channels^{12,25-28} which differ with respect to sensitivity to inorganic and organic blockers, ionic selectivity, and activation rate and voltage range, all were electrically excitable with strongly voltage-dependent gating. We suggest that the mitogen-regulated channel may represent a new class of voltage-independent Ca^{2+} channels.

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Opposite effects of cyclic GMP and cyclic AMP on Ca^{2+} current in single heart cells

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The slow inward Ca^{2+} current, I_{Ca} , is fundamental in the initiation of cardiac contraction and neurohormonal regulation of cardiac function¹. It is increased by β -adrenergic agonists, which stimulate synthesis of cyclic AMP (cAMP) and cAMP-dependent phosphorylation²⁻⁴. The neurotransmitter acetylcholine reduces I_{Ca} ⁵⁻⁷ by an unknown mechanism^{8,9}. There is strong evidence that acetylcholine reduces I_{Ca} by decreasing adenylate cyclase activity⁷, but cGMP has also been implicated as ACh stimulates cGMP accumulation and activates cGMP-dependent protein kinase¹⁰⁻¹³. Application of cGMP decreases contractile force¹⁴, decreases ^{45}Ca flux¹⁵, shortens the duration of action potentials¹⁶ and inhibits Ca-dependent action potentials¹⁷. Other studies, however, have concluded that cGMP levels do not correlate with contractile force^{10,11,18,19} and that cGMP has no effect on I_{Ca} ²⁰. We have therefore examined the effects of intracellular perfusion of cGMP on I_{Ca} using isolated, voltage-clamped cells from frog ventricle. We find that cGMP has negligible effects on basal I_{Ca} , but greatly decreases the I_{Ca} that had been elevated by β -adrenergic agonists or by intracellular perfusion with cAMP. The decrease of I_{Ca} is mediated by cAMP hydrolysis via a cGMP-stimulated cyclic nucleotide phosphodiesterase.

Single cells from frog ventricle were voltage-clamped with the whole-cell patch clamp technique as described previously⁷. To block K^+ currents, the patch pipette contained an intracellular medium with 120 mM Cs and the cells were superfused with 20 mM Cs. The fast Na^+ current was blocked with tetrodotoxin (TTX). Under these conditions, depolarizing pulses from -80 to 0 mV elicit an inward Ca current that can be blocked completely by 500 μ M Cd^{2+} .

Figure 1 shows the relationship between the concentration of the β -adrenergic agonist isoprenaline and I_{Ca} amplitude in two groups of cells: one group perfused with normal intracellular medium (circles); and the other with intracellular medium containing 20 μ M cGMP (squares). The shapes of the dose-response curves are the same for both groups of cells, but the increase in I_{Ca} by isoprenaline is considerably smaller in the cells perfused with cGMP. The cGMP has no effect on control I_{Ca} in the absence of isoprenaline or at isoprenaline concentrations < 100 nM.

To clarify the effect of cGMP, we used a perfused patch pipette to enable the solution inside the cell to be changed during an experiment. When the cell is bathed in control Ringer's solution, intracellular perfusion with 20 μ M cGMP has negligible effect (Fig. 2a). When cGMP is washed out and the perfusion is switched to a medium containing 5 μ M cAMP, I_{Ca} increases ~tenfold. The lag period of several minutes between changing the perfusion and the effect was largely caused by the dead volume of the perfusion system. In 8 experiments using a perfused pipette, 20 μ M cGMP had no effect on basal I_{Ca} (I_{Ca} in cGMP was $104 \pm 18\%$ of control I_{Ca} , mean $\pm$ s.d.). In contrast, when the cells are bathed in 3×10^{-7} M isoprenaline, 1 μ M cGMP produces a rapid decrease in I_{Ca} (Fig. 2b). The response to cGMP is dose-dependent with a threshold in the submicromolar range: 0.3 μ M cyclic GMP decreases cAMP-elevated I_{Ca} by $23 \pm 18\%$ ($n = 4$). Micromolar concentrations produce the

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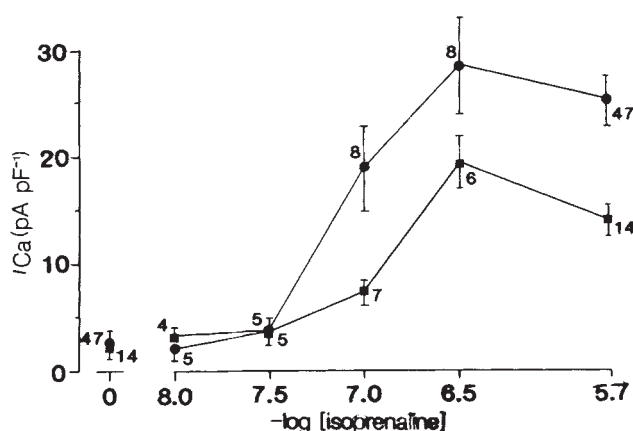


Fig. 1 Effect of intracellular perfusion with cGMP on isoprenaline dose-response curve. Cells were positioned at the end of one of six capillary tubes (internal diameter = 250 μ m) containing different isoprenaline concentrations⁷. The time required for moving the cell from one capillary to another was <10 s. I_{Ca} was allowed to stabilize for at least several minutes before moving to a new solution. Isoprenaline concentrations were tested without returning to control Ringer. Cells were perfused with either control intracellular medium (circles) or medium containing 20 μ M cGMP (squares). Isoprenaline concentrations were not tested in all cells, but in 5 control cells and 4 cGMP cells recorded on the same day, complete dose-response curves were obtained and the results were statistically the same as those shown for the pooled experiments. I_{Ca} was normalized to cell membrane capacitance. Error bars, $\pm$ s.d. Numbers indicate numbers of cells.

Methods. Control Ringer's solution contains 88.4 mM NaCl, 20 mM CsCl, 23.8 mM NaHCO₃, 0.6 mM NaH₂PO₄, 1.8 mM MgCl₂, 1.8 mM CaCl₂, 5 mM sodium pyruvate, 5 mM glucose and 3×10^{-7} TTX, pH 7.4 by gassing with 95% O₂, 5% CO₂. Control intracellular medium contained 120 mM CsCl, 5 mM EGTA, 4 mM MgCl₂, 5 mM Na₂CP, 3 mM Na₂ATP, 0.4 mM Na₂GTP, 10 mM HEPES, pH 7.1 with KOH. Cells were enzymatically dispersed from frog (*Rana esculenta*) ventricle and I_{Ca} recorded using the whole-cell patch-clamp technique. Resistance of patch electrodes = 1–3 M Ω . After disruption of the membrane patch, membrane capacitance of the whole cell and series resistance were measured and compensated when necessary. Series resistance was usually <5 M Ω of which 50–90% could be compensated. Holding potential, -80 mV. In all experiments, the cell was depolarized every 8 s from -80 to 0 mV for 200 ms to elicit I_{Ca} . I_{Ca} was measured as the difference between peak inward current and the current at the end of the 200-ms pulse.

maximum effect: 1 μ M cGMP produces a $40 \pm 16\%$ ($n=6$) decrease and 20 μ M cGMP a $50 \pm 11\%$ ($n=4$) decrease in I_{Ca} .

To determine whether cGMP exerts its effect at a site before or after cAMP synthesis, we tested the effect of cGMP on I_{Ca} elevated by intracellular perfusion with cAMP (Fig. 2c). We found that 5 μ M cAMP increases I_{Ca} almost 13-fold and addition of 20 μ M cGMP to the cAMP-containing medium causes a rapid reduction in I_{Ca} . In 17 cells, 20 μ M cGMP reversibly decreases the cAMP-elevated current $63 \pm 24\%$.

The effect of cGMP on isoprenaline- or cAMP-stimulated I_{Ca} is specific to 3':5' cGMP (Table 1). Two experiments show that hydrolysis of cGMP is not necessary for its effect (Table 1): 100 μ M M&B22948, a selective inhibitor of cGMP phosphodiesterase²¹, has no effect on the response to cGMP. Furthermore, 5'-GMP is several hundredfold less effective than cGMP in reducing cAMP-elevated I_{Ca} : 20 μ M 5'-GMP has no effect and even 200 μ M 5'-GMP has only a small, slow effect (Fig. 3a).

To determine whether the effect of cGMP is mediated by cyclic GMP-dependent protein kinase, we studied the effectiveness of analogues of cyclic GMP in reducing I_{Ca} . The cGMP does not act through the protein kinase because 8-bromo-cGMP, which is $\sim 5\times$ more potent than cGMP in activating the protein kinase²², is ineffective in reducing I_{Ca} (Fig. 3b; Table 1).

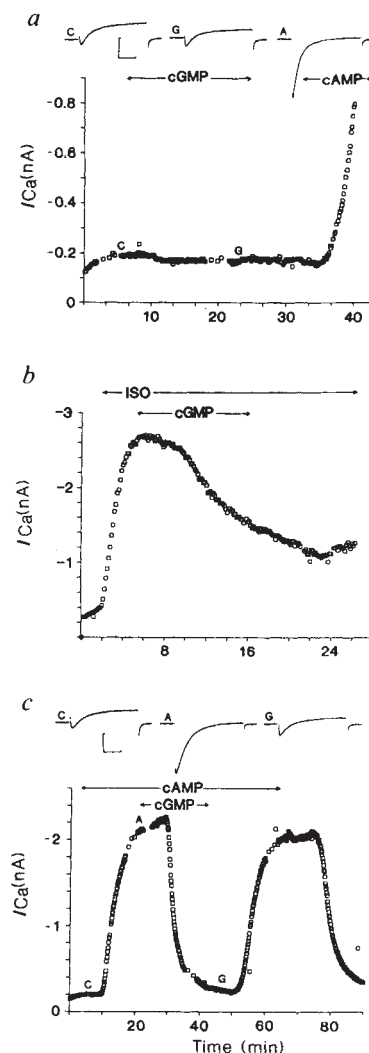
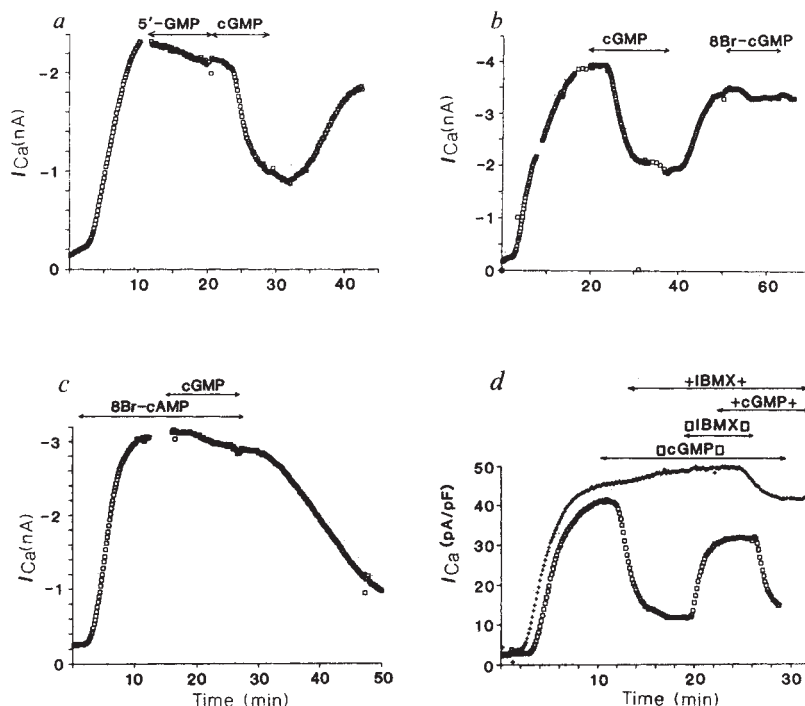


Fig. 2 Effects of intracellular perfusion of cGMP on I_{Ca} in an isolated ventricular cell. **a**, Effect of intracellular cGMP on basal I_{Ca} . The cell was exposed to control Ringer's solution for the entire experiment and internally perfused with control intracellular medium except for the periods marked. cGMP, control medium plus 20 μ M cGMP; cAMP, control medium plus 5 μ M cAMP. **b**, Effects of intracellular cGMP on I_{Ca} stimulated by 0.3 μ M isoprenaline. The cell was exposed to control Ringer's solution for the first 1.7 min of the experiment and with Ringer's solution containing 0.3 μ M isoprenaline during the period marked ISO. The cell was internally perfused with either control medium or medium containing 1 μ M cGMP. **c**, Effect of intracellular perfusion of cAMP and cGMP on I_{Ca} . The cell was exposed to control Ringer's solution for the entire experiment and internally perfused with control intracellular medium except for the periods indicated. cAMP, control medium plus 5 μ M cAMP; cGMP, 20 μ M cGMP added to the cAMP-containing medium. Current traces above **a** and **c** were recorded at the times indicated by the corresponding letters on the graphs. C, control; A, cAMP; G, cGMP. Calibration, 50 ms; C and G, 200 pA; A, 800 pA. Current traces were recorded and replayed from magnetic tape at 3–3/4 inches per second (i.p.s.) and digitized at 5 kHz. Each trace is the mean of three sequential, digitized records.

Methods. The perfused patch pipette was modified only slightly from that described by others⁴. A fine polyethylene capillary tube, pulled from a 0.3-mm internal diameter, 0.7-mm outside diameter catheter, was inserted into the patch pipette within 0.3–0.6 mm of the tip. The distal end of the capillary was placed into reservoirs of different solutions. The pipette was perfused by applying negative pressure to the pipette, which aspirated the solution from the capillary. Perfusion was maintained constant throughout the experiment, except for <30 s while changing solutions.

Fig. 3 Effects of guanine nucleotides and IBMX on I_{Ca} . *a*, Effect of 200 μ M 5'-GMP on I_{Ca} . Internal perfusion with 5 μ M cAMP began at time 0 and continued for the duration of experiment. 200 μ M 5'-GMP or 20 μ M cGMP was added to the cAMP-containing perfusion for the periods shown. *b*, Effect of 8-bromo-cGMP on I_{Ca} . Perfusion with 5 μ M cAMP began at time 0 and cGMP (1 μ M) or 8-bromo-cGMP (20 μ M) was added during the periods shown. *c*, Effect of cGMP on I_{Ca} elevated by 8-bromo-cAMP. The cell was perfused with normal intracellular medium except for the periods marked: 8-bromo-cAMP (1 μ M); cGMP (20 μ M) + 8-bromo cGMP. Proper function of the perfusion was demonstrated by the decrease in I_{Ca} on washing out the 8-bromo-cAMP. Similar results were obtained with 5 μ M 8-bromo-cAMP. *d*, Effects of IBMX on I_{Ca} . Two different cells are shown with I_{Ca} normalized to cell capacitance. In both cells, perfusion with 20 μ M cAMP began at time 0 and continued for the duration of the experiment. Crosses (upper curve): the cell was perfused internally and also superfused with 100 μ M IBMX for the period marked (+ IBMX+). I_{Ca} increased ~10%. Subsequent perfusion with 20 μ M cGMP (+cGMP+) produces only a 20% diminution in I_{Ca} . Squares (lower curve): perfusion with 20 μ M cGMP for the period shown ($\square$ cGMP $\square$) produced a 75% reduction in I_{Ca} . Subsequent addition of 100 μ M IBMX to the perfusion and superfusion media for the period marked ($\square$ IBMX $\square$) produces a large increase in I_{Ca} .



The cGMP effect is probably mediated by the cGMP-stimulated cyclic nucleotide phosphodiesterase^{11,13,21,23}, a major receptor for cGMP in various cells. Two experiments support the hypothesis that the effect of cGMP on I_{Ca} is mediated by this phosphodiesterase: cGMP has no effect on I_{Ca} stimulated by the phosphodiesterase-resistant 8-bromo-cAMP (Fig. 3c); and the phosphodiesterase inhibitor 3-isobutyl 1-methyl xanthine (IBMX), which inhibits the cGMP-stimulated phosphodiesterase²¹, partially reduces or reverses the effect of cGMP (Fig. 3d, Table 1). IBMX (100 μ M) has no effect on control I_{Ca} and has only a small effect on I_{Ca} stimulated by cAMP (Fig. 3c, upper curve, crosses; Table 1).

The hypothesis that cGMP is involved in regulation of cardiac contractility has lost favour partly because of difficulties in demonstrating specific cGMP-dependent phosphorylation in heart^{10,11,18,19}. The discovery of cGMP-binding proteins other than the protein kinase suggests that cGMP does not always act through a cGMP-dependent phosphorylation reaction. In photoreceptors, for example, cGMP regulates cation conductance through a phosphorylation-independent mechanism^{24,25}. Because the cGMP-stimulated cyclic nucleotide phosphodiesterase is found in many tissues^{11,23} stimulation of cAMP hydrolysis may be a common mechanism of cGMP action. If so, this would provide at least a partial molecular explanation for the hypothesis that cGMP and cAMP have opposing effects¹⁹.

Table 1 Effect of guanine nucleotides and IBMX on I_{Ca}

Treatment (μ M)	cAMP-elevated I_{Ca} (%) mean $\pm$ s.d. (no. of cells)
3':5'-cGMP (20)	37 $\pm$ 24 (17)
2':3'-cGMP (20)	100 $\pm$ 14 (4)
2':3'-cGMP (200)	91 $\pm$ 3 (2)
3':5'-cGMP (20) + M&B22948 (100)	33 $\pm$ 18 (5)
5'-GMP (20)	98 $\pm$ 8 (3)
5'-GMP (200)	84 $\pm$ 7 (4)
8-bromo-3':5'-cGMP (20)	92 $\pm$ 12 (10)
IBMX (100)	104 $\pm$ 0 (2)
3':5'-cGMP (20) + IBMX (100)	74 $\pm$ 17 (8)

I_{Ca} was increased by intracellular perfusion with 5 μ M cAMP before perfusion with the drugs listed.

From our experiments, however, it remains unclear whether cGMP stimulation of the phosphodiesterase decreases the steady-state levels of cAMP locally (for example, by poor local buffering of cAMP concentration by the pipette) or whether cAMP hydrolysis itself provides the regulatory signal (by release of free energy or hydrolytic products)²⁶.

The fact that acetylcholine is incapable of reducing I_{Ca} that has been elevated by perfusion with cAMP⁷, whereas cGMP can, suggests either that acetylcholine does not regulate I_{Ca} via cGMP or that acetylcholine-stimulated cGMP production is inhibited in our experiments. We favour the latter possibility because the presence of EGTA and ATP in our intracellular medium should inhibit guanylate cyclase, and it has been shown that cGMP levels can be increased by acetylcholine¹⁰⁻¹³.

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Weak acids may act as teratogens by accumulating in the basic milieu of the early mammalian embryo

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Among the eleven drugs or chemicals which are well-documented human teratogens, eight (or their main metabolites) are weak acids whereas none is a weak base. Moreover, 23 out of 32 acids tested have been found to be teratogenic in at least one animal species¹. The acidic property of drugs may therefore be an important determinant of teratogenicity. We demonstrate here that the intracellular pH (pH_i) of the mouse and rat embryo is higher than that of maternal plasma, as determined by the relative accumulation of dimethadione. The antiepileptic drug valproic acid and its pharmacologically active unsaturated metabolite accumulate in embryonic tissue to higher concentrations than in maternal plasma, whereas the essentially neutral amide of valproic acid (valpromide) or ethosuximide do not accumulate in the embryo; we further demonstrate in the rat that the pH_i of the embryo decreases with advancing gestation; in general agreement with the pH partition hypothesis, the exposure of the embryo to valproic acid also decreases significantly during that period. Furthermore, the amides of two weak acid teratogens, valpromide and methoxyacetamide, and the imide ethosuximide, are much less teratogenic than their acid counterparts. Our results suggest that weakly acidic drugs, by virtue of their physico-chemical nature, accumulate in the early embryo with its relatively high pH_i .

The intracellular pH of 9-day-old CD1 mouse embryos (vaginal plug = day 0) was determined by maternal intraperitoneal (i.p.) injection of 100 $\mu\text{Ci kg}^{-1}$ of ^{14}C -dimethadione (5,5'-dimethyloxazolidine-2,4-dione, or DMO, the demethylated metabolite of trimethadione; specific activity 50 mCi mmol⁻¹) to which 50 mg kg⁻¹ of cold DMO had been added. Two hours

after injection, when DMO has reached transplacental equilibrium, the embryos were removed from the uterus and a maternal blood sample was taken from the abdominal aorta. The pH of maternal blood was determined and the ^{14}C content of maternal plasma and embryo homogenate determined by liquid scintillation spectrometry. Other mice were killed on day 9 of pregnancy and the chloride concentration of the embryo and maternal plasma determined as a means of measuring the extracellular space ($59.66 \pm 2.56\%$). Embryonic pH_i was then calculated using the formula of Waddell and Butler². Similar procedures were used to determine pH_i of day 11 and day 14 rat embryos.

Embryonic pH_i (7.64 ± 0.07) in the mouse was about 0.4 units higher than maternal plasma pH (7.26 ± 0.03) on day 9 of gestation (see Table 2). This is a considerable difference, which should lead to a significant accumulation of weak acids at the site of more basic pH. We examined this possibility by determining the transplacental distribution of valproic acid, chosen because it is a weak acid (pK_a 4.7), a human^{3,4}, mouse^{5,6}, monkey²² and rat⁷ teratogen, and because analytical methodology is of sufficient sensitivity for measurements in small amounts of tissue⁸. Following i.p. administration of a single teratogenic dose in the mouse on day 9 of gestation, embryo concentrations of valproic acid exceeded corresponding free (unbound) maternal plasma levels by a factor of 2–2.8. The accumulation in the embryos was highly significant at all injection–analysis time intervals examined. Because i.p. administration in experimental animals could result in the direct transfer of drug into the embryonic compartment (before it reaches the systemic circulation), we also investigated alternative routes of application. Both subcutaneous injection in the mouse on day 9 (Table 1) and oral intubation in the rat on day 11 (Table 2) produced significantly higher embryonic concentrations than maternal plasma levels.

If the pH gradient between maternal plasma and embryo is to be a major determinant for the extent of placental transfer, several requirements must be fulfilled.

(1) Several acids should accumulate in the embryo. Indeed, the main pharmacologically active metabolite of valproic acid—2-propyl-2-pentenoic acid—also accumulated in the mouse embryo on day 9 of gestation (Table 1), although to a lesser degree than valproate. Rats showed a doubling in the accumula-

Table 1 Placental transfer during mouse organogenesis of two weakly acidic drugs and two related substances with low ionizing potential

				Concentration (mean $\pm$ s.d.)			E/P [†] ratio (mean $\pm$ s.d.)
Substance		Time interval*	<i>n</i>	Maternal plasma ($\mu\text{g ml}^{-1}$)		Embryo proper ($\mu\text{g g}^{-1}$)	
				Total	Free		
Valproic acid (2-propylpentanoic acid)	i.p.	0.5	4	369 $\pm$ 54	220 $\pm$ 35	437 $\pm$ 42	1.99
	i.p.	1	5	269 $\pm$ 46	144 $\pm$ 38	402 $\pm$ 243	2.79
	s.c.	0.5	5	474 $\pm$ 41	331 $\pm$ 22	549 $\pm$ 144	1.65 $\pm$ 0.40
	s.c.	1	5	371 $\pm$ 117	273 $\pm$ 76	630 $\pm$ 62	2.29 $\pm$ 0.24¶
2-Propyl-2-pentenoic acid	s.c.	0.5	4	607 $\pm$ 112	362 $\pm$ 68	442 $\pm$ 136§	1.24 $\pm$ 0.37
	s.c.	1	4	519 $\pm$ 105	279 $\pm$ 40	427 $\pm$ 64	1.50 $\pm$ 0.37
Valpromide (valproic acid amide)	s.c.	0.5	4	No binding‡	90 $\pm$ 20	88 $\pm$ 21§	0.98 $\pm$ 0.10
	s.c.	1	4		115 $\pm$ 18	116 $\pm$ 20	1.0 $\pm$ 0.09
Ethosuximide (2-ethyl-2-methyl-suxinimide)	s.c.	0.5	4	No binding‡	520 $\pm$ 87	520 $\pm$ 50	0.99 $\pm$ 0.016

NMRI mice received a single dose of 300 mg of the drugs per kg body weight i.p. or 400 mg kg⁻¹ subcutaneously (s.c.) on the morning of day 9 of gestation. Maternal plasma was prepared from arterial blood and the embryo proper was isolated (average number of somites was 15) and pooled for each litter. Drug concentrations in maternal plasma and embryo homogenate (prepared after dilution with water at 0 °C with ultrasonication) were determined by gas chromatography–mass spectrometry⁸. The free drug concentration in maternal plasma was determined following ultrafiltration through Amicon YMT membranes.

* Between injection and analysis.

† Embryo proper/free maternal plasma concentration ratio.

‡ Plasma protein binding lower than 10%.

§ Concentrations of valproic acid (as metabolite) in the embryo were always less than 1% of corresponding concentrations of the administered drug.

|| Significantly higher than the corresponding value for valpromide ($P < 0.05$).

¶ Significantly higher than the corresponding value for valpromide ($P < 0.001$).

Table 2 Comparison of the placental transfer of valproic acid on day 11 and day 14 of gestation in the rat

Time interval* (h)	Embryo proper/free maternal plasma concentration ratio (means $\pm$ s.d.; $n = 4$)		<i>P</i>
	Day 11	Day 14	
0.5	1.35 $\pm$ 0.13	0.94 $\pm$ 0.08	<0.005
1	1.42 $\pm$ 0.50	1.08 $\pm$ 0.04	NS
3	1.38 $\pm$ 0.22	0.59 $\pm$ 0.20	<0.005
Maternal plasma pH	7.44 $\pm$ 0.04	7.47 $\pm$ 0.05	NS
Embryo pH _i	7.61 $\pm$ 0.08	7.05 $\pm$ 0.08	<0.005

Rats received a single dose of 200 mg of sodium valproate per kg body weight on either day 11 or day 14 of gestation. Analysis of maternal plasma (free and total) and embryos (pooled for each litter) was performed as described in Fig. 1 legend. The free fraction of valproic acid in maternal plasma (fraction not bound to proteins) was approximately half the corresponding value in the mouse. NS, not significant.

* Between injection and analysis.

tion of methoxyacetic acid, the teratogenic metabolite of methoxyethanol^{9,10} (data not shown). DMO accumulation in rat and mouse embryos was used to calculate the embryonic pH_i. A thalidomide metabolite, a glutamic acid derivative, has been found to accumulate in the rabbit blastocyst¹¹, and the authors speculated that thalidomide entered the embryo, was hydrolysed there (hydrolysis was expected to be considerable because of the relatively high pH (9) of the blastocoelic fluid) and the resulting acidic metabolite, because of its high polarity, became 'trapped'. It is entirely feasible that this acid metabolite accumulated in the embryo because of the pH partition hypothesis. Lastly, following administration of labelled halothane, accumulation of non-volatile radioactivity was observed in the mouse embryo¹²; it is highly likely that this radioactivity corresponded to the main metabolite, trichloroacetic acid.

(2) Accumulation should be dependent on the *pK_a* of the acid. Indeed, higher accumulation was found for valproic acid (*pK_a* 4.7) than for DMO (*pK_a* 6.13); ethosuximide (*pK_a* 9.2) and valpromide, both essentially non-ionized in the physiological pH range, reached similar concentrations in maternal plasma and embryo (Table 1).

(3) After distribution equilibrium has been reached, there should be a limited dependence of embryo/maternal plasma ratios on the time interval between drug application and sampling. This was found to be true for the transfer of valproic acid to the mouse embryo on day 9 and rat embryo on day 11 (Table 2). The slight rise of these ratios with increasing time is probably due to the extremely rapid clearance of valproic acid from maternal plasma (half-life < 1 h)⁵ which may exceed the rate of transport across the placental membranes. Such considerations may become particularly important if the embryo constitutes a 'deep compartment' for a drug with slowed rates of back-exchange with maternal plasma.

(4) The accumulation should depend on the pH gradient, that is, the embryo exposure should diminish with advancing gestation because of the decreasing pH_i of the embryo (Table 2). We investigated the placental transfer of valproic acid in the rat on day 11, when embryo pH_i exceeds maternal plasma pH, and on day 14, when embryo pH_i is lower than maternal plasma pH (samples provided by T. Mast and A. Hendrickx, California Regional Primate Center, Davis) and found that the embryo/free maternal plasma concentration ratios of valproic acid were indeed significantly higher on day 11 than on day 14 (Table 3). Salicylate was also found to accumulate in rat embryo on day 10 of gestation, but not during later gestational stages (days 13 and 17)¹³.

Table 3 Teratological effects of valproic acid and methoxyethanol compared with their amides and ethosuximide

Agent	Dose	No. of implants	% Resorption	% Survivors with exencephaly
Methoxyethanol*	400	76	25	49
Methoxyacetamide	400	133	9	0
Na-valproate	600	136	45	61
Valpromide	600	116	25	1
Ethosuximide	600	100	6	0
Control	Water	165	7	0

NMRI mice received aqueous solutions of the agents i.p. on day 8 of pregnancy (plug date = day 0). The mice were killed on day 18 and the fetuses examined for the presence of gross structural malformations, especially exencephaly.

* Teratogenesis following methoxyethanol administration is due to the oxidized metabolite, methoxyacetic acid^{9,10}.

(5) Accumulation in the embryo should depend on an ionizable hydrogen. If the acid function of valproic acid was transformed into an essentially neutral amide group, the resulting drug, valpromide, did not accumulate in the embryo and embryo levels of this drug were very similar to those in maternal plasma (Table 1). Comparable results were also obtained for ethosuximide (Table 1), a drug possessing an imide function of high *pK_a* (9.3) which is also essentially non-ionized at physiological pH.

Interestingly, Willhite and Shealy¹⁴ demonstrated that the amides of all-*trans* and 13-*cis*-retinoic acid were much less teratogenic than the corresponding acids in hamster embryos, pointing to the importance of the carboxyl group. We pursued this idea by comparing the ability of the amides of valproic acid and methoxyacetic acid to induce exencephaly when administered to NMRI mice on day 8 of pregnancy. In agreement with the hypothesis, we found that changing the chemical structure of weak acid teratogens by amidation almost abolished their teratogenicity (Table 3).

Valpromide concentrations, however, were relatively low in this study (Table 1) and the high distribution volume of this drug prevented a direct comparison of the teratogenic potencies of valproic acid and valpromide. The high maternal toxicity of valpromide in the mouse¹⁵ also prevented us from increasing the dose further. Instead, we used ethosuximide, an alternative drug to valproic acid in clinical practice. The structure of ethosuximide differs from that of valproic acid, but resembles that of DMO. Although the concentration of ethosuximide in the embryo was similar to that of valproic acid, teratogenicity was not observed. Sullivan and McElhatton¹⁶ and Fabro *et al.*¹⁷ have reported that ethosuximide is only marginally teratogenic. Interestingly, 5-propyl-2-pentenoic acid, despite accumulating in high embryo concentrations, was not teratogenic in the mouse¹⁸. There is thus a strict structural requirement for the expression of teratogenesis. Indeed, a study of metabolites and analogues of valproic acid revealed that, as with thalidomide¹⁹, the teratogenicity of this class of compounds is a much more specific property than pharmacological activity²⁰. Thus, acidity may be an important, but not a sufficient, property for a drug to exert its teratogenicity.

Transplacental pharmacokinetics are determined by various parameters²¹. The rate of transfer depends on lipid solubility and the relative molecular mass of the drug as well as on placental blood flow and structure, while the extent of transfer depends on protein binding of the drug on each side of the placenta, active transport mechanisms, the presence of 'deep compartments' and the degree of drug ionization. We have demonstrated here that the latter aspect is of paramount importance for the transplacental distribution of acidic drugs during the critical phase of organogenesis because of the alkaline milieu of the early embryo. If our hypothesis is correct it should provide insight into the mechanisms of teratogenesis.

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Maternal control of *Drosophila* segmentation gene expression

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Several genes have been identified that are involved in establishing the segmented body pattern during development of the fruit-fly *Drosophila melanogaster*. These fall into several classes on the basis of the kind of alteration to the wild-type segmentation pattern observed in mutant embryos. For example, mutations of the pair-rule¹ class, such as *fushi tarazu* (*ftz*)¹⁻³, cause the deletion of pattern elements with a two-segment periodicity; those of the gap class¹, such as *knirps*^{1,4}, cause the deletion of contiguous groups of segments. The availability of antibodies against the *ftz* protein has allowed its spatial pattern of expression to be studied during the development of wild-type⁵ and mutant⁶ embryos. The aim of the latter kind of experiment is to investigate possible interactions between these important genes. We have recently reported that *knirps* mutations cause a striking alteration to the pattern of transverse stripes of *ftz* expression usually seen during embryogenesis⁶. *Knirps* is a zygotically-expressed gene, but recently a class of maternally-active genes has been identified that causes similar defects in pattern formation⁷⁻⁹. We have now investigated the pattern of *ftz* expression in mutants of this class and have found that while they do have features seen in *knirps* mutants, they also exhibit significant differences between the different mutations reflecting the distinct but overlapping domains of gene activity. These observations demonstrate that maternally-active segmentation genes regulate zygotic gene expression, and that some of their effects on *ftz* may be directed through the *knirps* gene.

In large-scale genetic screens for maternal genes required for normal embryonic pattern formation⁷⁻⁹, at least five (*vasa*, *valois*,

staufer, *tudor* and *oskar*) have been identified which are required for normal abdominal segmentation. Embryos produced by females homozygous for mutations in any of the five genes exhibit deletions of abdominal segments that resemble, to various degrees, defects observed in embryos homozygous for mutations of the zygotically-active gap locus *knirps*^{1,4} (Fig. 1). The abdominal deletions caused by the maternal-effect mutations are somewhat variable; the largest deletions are caused by mutations in *vasa* and *oskar*. In *vasa* and *oskar* embryos, all the abdominal segments are deleted and the thorax appears juxtaposed to very posterior structures including the telson and posterior spiracles⁷ (Fig. 1b,f). Mutations in the other three genes usually leave the abdominal denticle belts corresponding to the first and eighth abdominal segments intact but delete and fuse the intervening abdominal segments to different extents. One particular deletion pattern, which can result from any of the five mutations, is a large field of abdominal denticles followed by a region of naked cuticle. The broad band of denticles, representing one enlarged abdominal segment, closely resembles the cuticle pattern formed by the zygotic segmentation mutation *knirps* (Fig. 1g), and was most frequently found in *valois* (Fig. 1c) and *staufer* (Fig. 1e). In addition to the abdominal deletions, *staufer* also causes deletions of head structures. Mutations in all five of the genes result in the absence of the germline precursor cells, the pole cells⁷⁻⁹.

To investigate how maternal genes may exert an influence on the zygotic segmentation program, antibodies have been used to localize the protein encoded by the *ftz*^{2,3,10,11} pair-rule segmentation gene in whole-mount mutant embryos. The *ftz* protein is normally expressed in seven transverse stripes of nuclei during cellularization of the embryo. These stripes correspond to parts of alternate double-segment units^{5,12}. A segment (or parasegment) primordium is about four cells across, in the anterior-posterior axis, at the blastoderm stage. At the beginning of gastrulation the *ftz* stripes are about three nuclei in width and are separated by an 'interstripe' of five relatively unstained nuclei (Fig. 2a). The posterior stripe is widest, remaining about five nuclei in width throughout germ-band elongation.

Each of the mutants has a distinctive pattern of *ftz* expression, but there are some common features exhibited by mutations in all of the five loci. The first (most anterior) two stripes of *ftz* expression are relatively normal in all five mutants (Fig. 2), and are found at the position of the primordia for the normal labial-maxillary and T1-T2 segments. These segments appear normal in larvae from mutant mothers (Fig. 1). The seventh stripe is also normal (Fig. 2) and corresponds to the posterior segmental region of A8-A10 which gives rise to the telson, a structure which forms normally in the embryos. The major aberrations occur in the region between the second and seventh *ftz* stripes. Either the second 'interstripe' (*vasa*), or the third stripe (*valois*, *tudor*), or both (*staufer*, *oskar*) appear(s) wider than in a normal embryo, and the fourth through sixth stripes become narrower or disappear altogether (Fig. 2b-f).

There are a number of significant differences between the effects of the different mutations. In embryos from homozygous *vasa*^{PD} mothers, *ftz* protein is present only as a weak, partial band in the central abdominal region (Fig. 2b). In embryos from homozygous *valois*^{RB} (Fig. 2c) and *tudor*¹ (Fig. 2d) mothers, *ftz* expression in the fourth, fifth and sixth stripes is restricted to fewer cells and the spaces between the stripes are narrowed. In the major pattern of *staufer* defects (90% of the embryos), there is a wide patch of *ftz* protein in the abdominal primordium and abnormal spacing of the two anterior stripes (Fig. 2e). However, in about 10% of the progeny of *staufer*^{HI} mothers, a *valois*-type pattern of *ftz* stripes is exhibited (Fig. 2f). In embryos from females homozygous for *oskar*¹⁶⁶, *ftz* expression is spread over a wide band of cells (Fig. 2f).

The cuticular phenotypes of these maternal effect mutants (Fig. 1b-f) resemble, to varying degrees, that of strong mutants of the gap locus *knirps*^{1,4} (Fig. 1g). Homozygous mutant *knirps*

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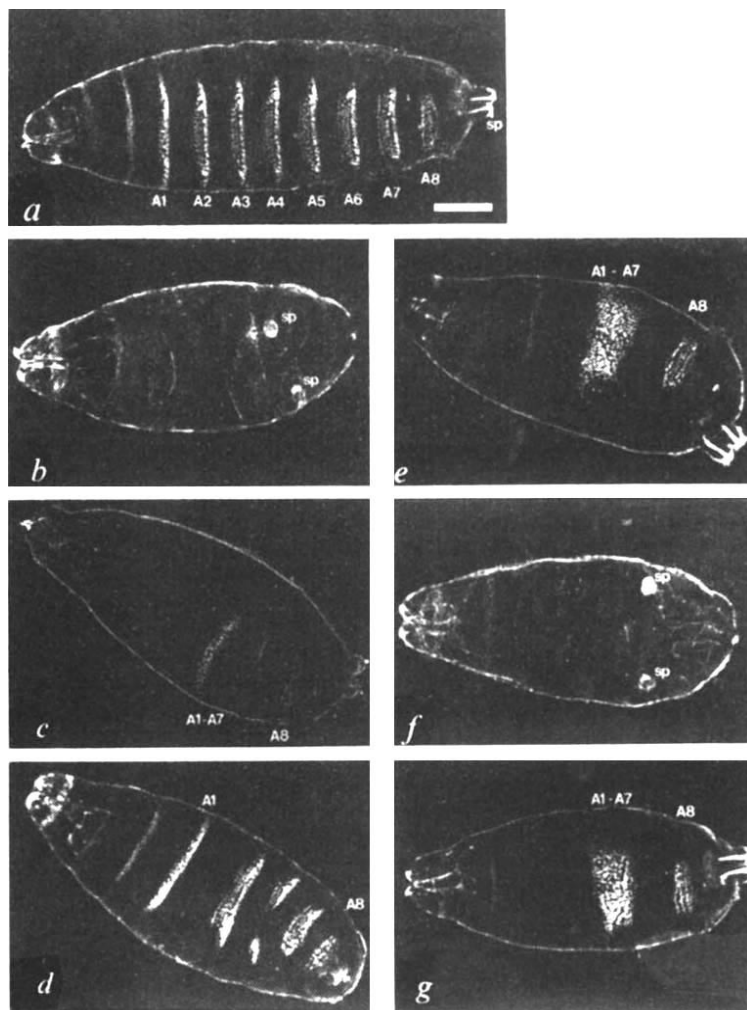


Fig. 1 The phenotypes of progeny of females homozygous for mutations at five loci controlling abdominal segmentation, and of embryos homozygous for *knirps*. *a-g*, Whole mounts of larval cuticles obtained from wild-type or homozygous mutant mothers, fixed and mounted as described by Van der Meer¹³. The identity and position of some abdominal denticle belts are indicated (A1-A8). *a*, Wild-type larva. Scale bar represents 100 μ m. *b*, *vasa*^{PD}. Most of the anterior abdominal segments are deleted; sp, spiracles. *c*, *valois*^{RB}. Segments A1-A7 are fused. *d*, *tudor*¹. Segments A2-A6 defective. *e*, *staufen*^{HL}. Segments A1-A7 fused. *f*, *oskar*¹⁶⁶. Most denticle bands are deleted; sp, spiracles. *g*, Homozygous *knirps*^{11E72} larva, obtained from a balanced heterozygous mutant stock. Segments A1-A7 fused.

embryos exhibit a band of *ftz* protein about 27 nuclei in width in the region normally comprising the third through sixth stripes (Fig. 2h). Since the absence of *knirps* gene activity leads to a uniform 'on' state of *ftz* across most of the abdominal region⁶, a primary defect of mutations at the *staufen* and *oskar* loci may be the failure to properly activate the *knirps* gene in certain parts of the embryo. The differences between the *ftz* patterns resulting from mutations in *knirps*, *staufen* or *oskar* could be due to differences in allele strengths or in the exact spatial limits of influence of the genes.

In *vasa* embryos, *ftz* expression is affected in a manner opposite to that of *staufen*, *oskar*, and *knirps* in that *ftz* protein is not produced in most of the anterior abdominal cells. Since *vasa* and *oskar*, for example, produce very similar larval phenotypes (Fig. 1b and f), quite different alterations in the *ftz* pattern can lead to similar terminal cuticular phenotypes. The *ftz* pattern suggests that *vasa* influences a different pathway of abdominal segmentation gene regulation or that *vasa* interacts with *knirps* differently than do the other genes.

Observed at the level of *ftz* expression, the maternal segmentation mutations result in a shift of cellular fates at the blastoderm stage: it appears that anterior regions become expanded at the expense of posterior regions. The common effect of all five mutations is the enlargement of certain anterior stripes and interstripes coupled with the increasingly substantial compression and eventual elimination of more posterior stripes. The particular stripes or interstripes which are enlarged vary between individual loci, indicating that the exact domains of influence of their products may differ.

The results also suggest that expansion of a given *ftz* stripe does not necessarily result in gross abnormalities in the differentiated cuticle, while compression does. In wild-type embryos, the third stripe of *ftz* expression marks the posterior part of the third thoracic and the anterior portion of the first abdominal segment. In spite of the fact that the third stripe is enlarged in blastoderm embryos derived from *valois*, *tudor*, *staufen*, *oskar* and *knirps*, the third thoracic segments of the larvae have normal cuticular morphology. The first abdominal denticle band also usually appears to be normal in many of the embryos with large cuticular deletions and is present, though abnormally large, in embryos with only one denticle field. These observations argue for the existence of some regulatory mechanism which can compensate to produce relatively normal segmental pattern when primordial cell pools are too large. By contrast, the compression of the *ftz* pattern leads to deletion of segments from the final cuticular pattern. It appears that within the region of pattern compression, the cells cannot differentiate their corresponding pattern elements and may therefore assume different fates or be eliminated. The fourth abdominal segment is most often deleted, followed by the fifth, and then the third and sixth abdominal segments⁷⁻⁹. In wild-type embryos at the blastoderm stage, the posterior part of segment A4 and the anterior part of segment A5 are represented by the fifth stripe of *ftz* expression. This stripe is in the middle of the compressed stripe region in the mutants. In the stronger mutants, the pattern compression is so extreme that, even at the blastoderm stage, no trace of the compressed stripes is visible and correspondingly all traces of abdominal segments 2-7 have disappeared from

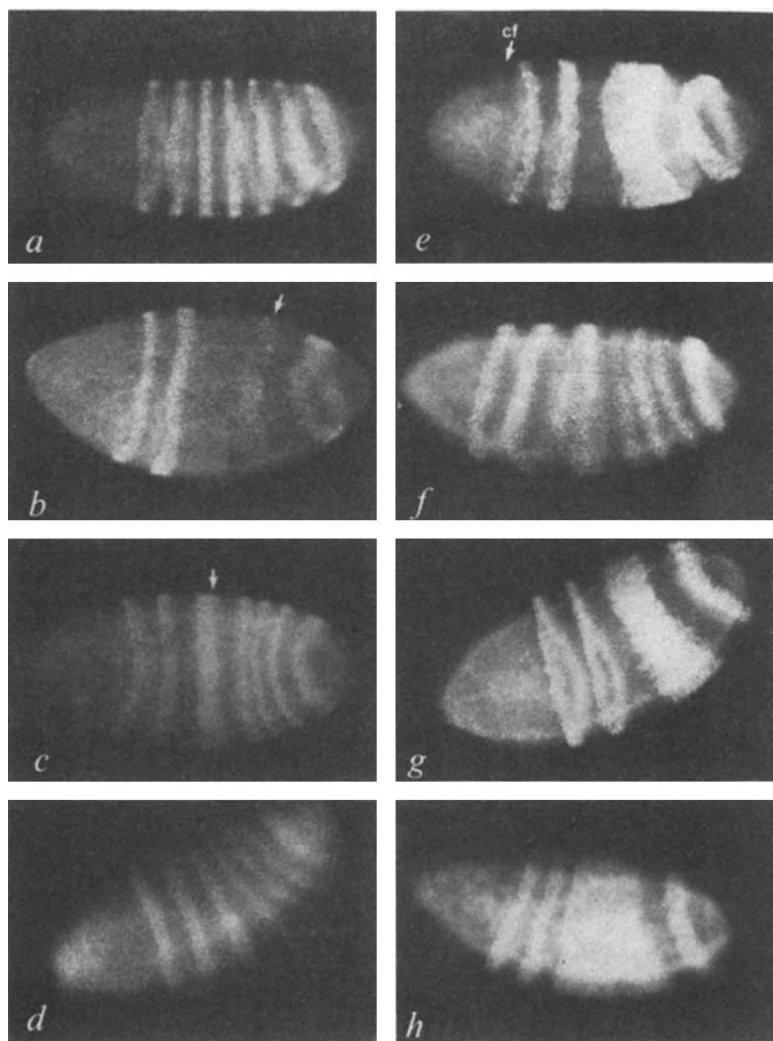


Fig. 2 Expression of the *ftz* protein in embryos from females homozygous for mutations in abdominal segmentation genes. In each photograph, the anterior end of the embryo is at the left, and the ventral side, when visible, is at the bottom. The alleles used were the strongest available, but may not be null alleles. *a-h*, Whole-mount embryos were collected at 25 °C and fixed, incubated with anti-*ftz* antibodies and stained with fluorescein conjugated goat anti-rabbit antibody⁵. *a*, A wild-type embryo just beginning gastrulation. The *ftz* protein stripes are roughly three nuclei wide except for the posterior stripe which is about five nuclei wide. *b*, *vasa*^{PD}: The enlarged second gap between the *ftz* stripes is followed by a region of weak *ftz* expression where the boundary between expressing and non-expressing cells is difficult to see (arrow). Often the band of weak *ftz* expression extends more anteriorly on the dorsal than on the ventral surface. *c*, *valois*^{RB}: The third stripe extends slightly farther to the posterior than in normal embryos, and the fourth, fifth, and sixth stripes are compressed. *d*, *tudor*: The second stripe is slightly wider (by about one cell) than in a wild-type embryo, whereas the first stripe is normal. The fourth, fifth and sixth stripes of *ftz* protein are narrow and more compressed than three normal stripes. *e, f*, *staufen*^{HL}: The two anterior stripes are spaced farther apart from each other than in wild-type embryos. Embryos from *staufen* mothers lack anterior head structures⁷, an alteration which is probably related to the anterior shift of the cephalic furrow (*e*, arrow) and the anterior shift of the first stripe of *ftz* protein. Posterior to the second stripe, the most common pattern observed (90% of the embryos) consists of an unstained region of about 7–9 nuclei followed by a third stripe that is 14–20 nuclei in width (*e*). In the second pattern (10% of the embryos), the wide band is split into four stripes, the overall pattern resembling that of *valois* embryos (*c*), but with a larger number of nuclei staining in each stripe (*f*). *g*, *oskar*¹⁶⁶: The first two stripes are farther apart than in wild-type embryos, but are not shifted anteriorly as in *staufen*. The third stripe in *oskar* consists of a broad band of *ftz* expression about 12–16 nuclei in width. The space between this band and the apparently normal posterior stripe is 3–5 cells wider than the space between the wild-type sixth and seventh stripes. *h*, Homozygous *knirps*^{IE72} embryo, exhibiting a wide band of *ftz* protein in the A1–A7 segment primordia. The first, second, and seventh *ftz* protein stripes are normal.

the cuticle.

We have previously shown that four zygotic gap genes (including *knirps*) and three of the seven pair-rule genes influence *ftz* expression, whereas four other pair-rule genes and three segment polarity genes have no effect⁶. These results place *ftz* in the middle of the zygotic segmentation gene hierarchy, with *ftz* acting after or in parallel to the seven zygotically-active genes that influence it, but before or independently of those that do not affect its expression. The maternal segmentation genes studied in this work appear to be at the top of the hierarchy, since they are expressed during oogenesis and alter *ftz* expression. It is likely that the different larval and molecular phenotypes of the maternally-active genes reflect the different effects of the maternal genes on other segmentation genes as well as on *ftz*. The identification of any direct regulatory interac-

tions between the maternally-active genes and *ftz* is complicated by the genetic hierarchy problem.

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